

SETAC EUROPE 35TH ANNUAL MEETING

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INNOVATION FOR TOMORROW: PROGRESS IN SAFE AND SUSTAINABLE CONCEPTS



ABSTRACT BOOK

Abstract Book

SETAC Europe 34th Annual Meeting

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This book compiles the abstracts from the 35th annual meeting of the Society of Environmental Toxicology and Chemistry – Europe (SETAC Europe), conducted from 11–15 May 2025 in Vienna, Austria.

The abstracts are reproduced as submitted by the author and accepted by the scientific committee. They appear in order of abstract code and alphabetical order per presentation type. The poster spotlight abstracts are included in the list of poster abstracts. The presenting author of each abstract is highlighted in bold.

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Society of Environmental Toxicology and Chemistry Europe (SETAC Europe)

About SETAC

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists, managers, engineers or others interested in environmental issues. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in North America in 1979 to fill the void and quickly saw dynamic growth in the Society's membership, meeting attendance and publications.

A unique strength of SETAC is its commitment to balance the scientific interests of government, academia and business. The Society by-laws mandate equal representation from these three sectors for officers of the World Council and Geographic Unit Boards of Directors and Councils, and in the composition of committees and other society activities. The proportion of members from each of the three sectors has remained nearly equal over the years.

The Society is concerned about global environmental issues. Its members are committed to Environmental Quality Through Science®, timely and effective communication of research, and interactions among professionals so that enhanced knowledge and increased personal exchanges occur. Therefore, SETAC publishes two globally esteemed scientific journals and convenes annual meetings around the world, showcasing cutting-edge science in poster and platform presentations. Because of its multidisciplinary approach, the scope of the science of SETAC is broader in concept and application than that of many other societies.

SETAC's growth is reflected in the founding of Geographic Units around the world. SETAC Europe was established in 1989 as an independent organisation, followed by SETAC Asia-Pacific in 1997 and SETAC Latin America in 1999. In 2002, the four existing organisations joined together under the governance of the SETAC World Council. SETAC Africa is the most recent Geographic Unit, which was adopted in 2012. As evidence of international acceptance of the SETAC model and of the great interest at the local level, regional chapters and branches have emerged in a number of countries.

SETAC publishes two journals, *Environmental Toxicology and Chemistry* (ET&C) and *Integrated Environmental Assessment and Management* (IEAM). ET&C is dedicated to furthering scientific knowledge and disseminating information on environmental toxicology and chemistry, including the application of these sciences to risk assessment. Integrated Environmental Assessment and Management focuses on the application of science in environmental decision-making, regulation and management, including aspects of policy and law, and the development of scientifically sound approaches to environmental problem solving. Together, these journals provide a forum for professionals in academia, business, government and other segments of society involved in the use, protection and management of the environment for the enhancement of ecological health and human welfare.

SETAC books provide timely in-depth reviews and critical appraisals on scientific subjects relevant to understanding a wide range of contemporary topics pertaining to the environment. These include any aspect of environmental chemistry, toxicology, risk assessment, risk management or environmental policy.

SETAC has two administrative offices, in Pensacola, Florida, USA, established in 1992, and in Brussels, Belgium, established in 1993.

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Environmental Quality Through Science®

Track 1. Environmental and Human Toxicology: From Molecules to Organisms, From Omics to in Vivo

1.01 Unveiling Long-Term Ecological Impacts: From Epigenetic Biomarkers to Multigenerational and Chronic Effects of Environmental Contaminants Including Their Mixtures

1.01.T-01 PFAS Across Generations: Developmental Exposure in Zebrafish Drives Behavioural, Transcriptomic and Epigenetic Disruptions

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Understanding how environmental pollutants impact organisms across generations is critical to addressing global ecological challenges. Perfluoroalkyl substances (PFAS), widely used in industrial and consumer products, pose significant risks due to their persistence and potential to induce heritable changes through epigenetic modifications. This study examines the multigenerational effects of perfluorooctane sulfonic acid (PFOS) and its replacement, perfluorobutane sulfonic acid (PFBS), on zebrafish, focusing on behavior, fertility, gene expression, and epigenetic alterations. While PFOS is notorious for its environmental toxicity and bioaccumulation, PFBS is less studied despite its growing use as an alternative. We exposed zebrafish embryos to environmentally relevant concentrations of PFOS, PFBS, or control conditions from 2 to 28 days post-fertilization, followed by a depuration period, after which the fish were raised in clean water. The exposed generation (F0) was analyzed for fertility, morphology, behavior, lipidomics, and transcriptomics, while the unexposed F1 and F2 generations were assessed for behavioral, transcriptomic, and DNA methylation changes. Our results revealed that PFOS exposure reduced fertility and adversely affected growth, organ development, and behavior in both larvae and adults of the F0, with sex-specific effects observed in behavior. PFBS also caused behavioral changes, albeit less severe. The transcriptomic analysis results showed disruptions in lipid and sugar metabolism, neurotransmission, and synaptic organization. Lipidomic findings indicated disruptions in hormone biosynthesis and fatty acid metabolism, explaining fertility reductions and behavioral alterations. Multi- and transgenerational effects were also observed on the F1 and F2 generations with behavioral changes persisting in both F1 and F2, correlating with gene expression modifications in the visual system and muscle function pathways. Epigenetic analysis identified DNA methylation changes in genes linked to neurodevelopment and neuronal function, providing evidence of heritable impacts of PFOS and PFBS. Our findings provide critical insights into the mechanisms by which PFAS exert long-term biological and ecological impacts, from immediate phenotypic changes to heritable epigenetic modifications. These results underscore the urgent need for further research and informed policy-making to mitigate the risks of PFAS exposure on ecosystems and public health.

1.01.T-02 Multigeneration Responses of *Daphnia magna* to Short Chain Per- and Polyfluorinated Substances (PFAS)

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Short chain per- and polyfluorinated substances (PFAS) have been widely used as replacements for long chain PFAS, and are therefore ubiquitously distributed in the environment. However, they remain largely unregulated owing to insufficient information on their environmental fate and effects. Moreover, due to their highly persistent and bioaccumulative nature, organisms living in contaminated ecosystems are chronically exposed to these short chain PFAS over multiple generations. Yet, in ecotoxicity experiments such chronic effects have barely been addressed, which are consequently overlooked in environmental risk assessment. Therefore, in this study, the ecotoxicity of perfluorobutane sulfonic acid (PFBS) and its precursor perfluorobutane sulfonamide (FBSA) to the aquatic invertebrate *Daphnia magna* was assessed under continuous exposure for six consecutive generations, following OECD guideline 211, with adult survival, reproduction, and population growth rate as endpoints. The observed effects were related to both the external (test medium) and internal PFAS concentrations in the daphnids. Over six generations, both PFBS and FBSA showed intensified ecotoxicity, increasing by 1.8 to 3.0, and 3.6 to 6.4 times, respectively. PFBS ecotoxicity increased as a result of elevated compound accumulation over generations, indicating a narcotic mode of action, whereas FBSA exerted specific reproductive toxicity, resulting in a more pronounced worsening of adverse effects over time. Compared to PFBS, FBSA was around 100 times more toxic in F0 (EC50_{reproduction} of 856 and 7.08 mg L⁻¹ for PFBS and FBSA, respectively),

escalating to over 435 times more toxic in F5 (EC50_reproduction of >478 and 1.10 mg L⁻¹ for PFBS and FBFA, respectively), and also showed a higher bioaccumulation potential (BAF of 7.76 and 16.4 L kg⁻¹ for PFBS and FBFA, respectively). These findings highlight that the conventional single-generation ecotoxicity tests underestimate PFAS ecotoxicity during multigeneration exposure, and that the environmental risks of PFAS cannot reliably be assessed by the current limited subset of studied compounds. This research was supported by the Open Technology Programme of the Dutch Research Council (NWO) [grant number 18725]

1.01.T-03 Multigenerational Reproductive Toxicity of Arsenic in Zebrafish (*Danio rerio*)

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Arsenic causes reproductive toxicity in animal models via endocrine disruption, however, whether the toxicity persists beyond one generation (F0) is largely unknown. Our study was designed to investigate whether the multigenerational reproductive effects of arsenic occur via maternal or paternal lineage and to gain insights into the potential underlying epigenetic mechanisms. Adult zebrafish (F0 generation) were exposed to environmentally relevant doses of arsenic via diet [0 (control), 30 (low), 60 (medium), and 100 (high) g/g dry weight, as arsenite] for 90 days. Following exposure, arsenic-treated females were crossed with control males, and vice versa, to assess the reproductive effects of sex-specific arsenic exposure. Arsenic exposure in females resulted in a dose-dependent decrease in fecundity and fertilization rate. In contrast, arsenic exposure in males induced similar reproductive effects predominantly at the high arsenic exposure dose. Subsequently, F1 generation larvae from different maternal and paternal arsenic treatments were raised to adulthood in clean water and diet, and males and females from the same treatment were then bred to evaluate the reproductive effects of maternal and paternal arsenic exposure. Maternal arsenic exposure decreased fecundity and fertilization success, even at the low dose, whereas the same reproductive effects were recorded only at medium and high doses with paternal arsenic exposure. In addition, the genes involved in the hypothalamus-pituitary gonad liver (HPGL) axis were consistently downregulated in F0 females directly exposed to arsenic as well as in F1 males and females maternally exposed to arsenic, irrespective of dose levels. On the other hand, the HPGL axis genes were downregulated in F0 males exposed directly to medium and high arsenic doses and in F1 generation males and females paternally exposed to the same arsenic doses. Furthermore, we also found that arsenic exposure led to hypermethylation of the promoter region of key HPGL axis genes, specifically steroidogenic factor-1 in the brain and cyp19a1a in the gonads in F0 and F1 males and females. This indicated that the downregulation of critical HPGL axis genes was mediated by arsenic-induced DNA hypermethylation. Overall, our study demonstrated that arsenic exposure causes multigenerational reproductive toxicity in zebrafish by disrupting the HPGL axis via epigenetic alterations, and these effects occur via both maternal and paternal lineages.

1.01.T-04 New Insights Into Cadmium Sub-Lethal Effects on *Daphnia magna*

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The rise in the production and use of electrical and electronic products leads to the increase in usage of cadmium, increasing the risk of exposures to this metal and justifying its inclusion in the list of restricted hazardous substances by the European Union. Despite the plethora of studies available, the full toxicity spectrum and the mechanisms behind cadmium toxicity in aquatic biota still need further clarification. The present study contributes to such clarification, by characterizing the sub-lethal effects of an exposure (7 days) to the EC20 concentration of cadmium (4.527 µg L⁻¹) in the freshwater keystone species *Daphnia magna*. A holistic approach across multiple levels of biological organization was applied, starting at a molecular level with epigenetic endpoints (total 5-mC DNA methylation and total DNA methyltransferases activity) and transcriptomic analysis (RNA-Seq), then working up to higher levels of biological organization by evaluating phenotypic effects at the sub-cellular and individual level. Exposure to cadmium caused global genome hypomethylation and altered activity of DNA methylation related enzymes. These changes in the epigenome were accompanied by significant gene transcription modulation, with 253 up- and 157 down-regulated genes, some of them in biological processes and pathways that correlate with the responses found for phenotypic endpoints. A significant increase of antioxidant enzymes, lipid peroxidation levels and DNA damage was recorded, alongside a reduction on physiological functions and somatic growth in exposed organisms. These findings provide insights into cadmium adverse outcome pathways considering DNA methylation as molecular initiating events. CESAM was funded by FCT/MCTES (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020) through national funds; project EPIBOOST, funded by the European Union (Grant 101078991; DOI:

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1.01.T-05 Beyond Exposure: Investigating the Transgenerational Effects of Bisphenol S in Zebrafish

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Bisphenol S (BPS), a commonly used substitute for Bisphenol A (BPA), has been detected in surface waters at increasing concentrations (from ng/L to low µg/L), primarily due to its widespread industrial applications, especially in the production of polycarbonate plastics and epoxy resins. As current knowledge regarding the toxicity of BPS in non-target organisms is limited, therefore, this study aims to evaluate the ecological risks of BPS in aquatic ecosystems through a transgenerational bioassay with *Danio rerio* (zebrafish) as a model organism. The first generation (F0) was exposed to environmentally relevant concentrations of BPS (0.4, 2, and 10 µg/L direct exposure) and subsequent generations, F1 and F2, were grown in clean water (intergenerational and transgenerational, respectively). We characterized physiological, biochemical and molecular responses to disclose cause-effects relationships and contribute to the understanding of the underlying mechanisms behind the adverse physiological responses. In F0, a significant decrease in the fertilization rate was observed and several biochemical biomarkers (i.e. catalase, glutathione S-transferase and acetylcholinesterase) were altered by the lowest and highest concentrations, in both males and females brains. Parental BPS exposure altered several endpoints in non-directly exposed zebrafish (F1 and F2), by affecting their growth, heart rate, behaviour and other physiological parameters such as Fulton's condition factor, gonadosomatic and hepatosomatic indexes. To better understand the observed effects described so far, we began by performing a transcriptomic analysis (RNA-seq) on F1 embryos at 24 hpf. F1 embryos descendant from parental exposure to 0.4 µg/L of BPS, were the ones that revealed the largest number of altered genes compared to the control group. The majority of those were downregulated and 8 KEGG pathways were affected, such as oxidative phosphorylation, RNA degradation, ribosome, cardiac muscle contraction and PPAR signalling pathway. Results so far suggest that BPS, at environmentally relevant concentrations, was capable of inducing several direct, inter and transgenerational effects in different biological processes in zebrafish. Therefore, with this conceptual approach, we also hope to promote the assessment of the environmental hazards and risks of this compound. This study is supported by the project TRANSEPIC (2022.02922.PTDC, doi: 10.54499/2022.02925.CEECIND/CP1728/CT0004) and the author Marta Ribeiro acknowledges the Portuguese Fundação para a Ciência e Tecnologia for her PhD grant (2022.12763.BD)

1.01.P Unveiling Long-Term Ecological Impacts: From Epigenetic Biomarkers to Multigenerational and Chronic Effects of Environmental Contaminants Including Their Mixtures

1.01.P-Tu001 Sub-lethal Effects of Cadmium and Ciprofloxacin in Freshwater Green Microalgae: Epigenetic and Phenotypic Responses

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Anthropogenic activities often lead to contamination of waters with harmful substances such as metals, pharmaceuticals and other exogenous chemicals. As epigenetic mechanisms shape gene-environment interactions, the study of the epigenetic alterations in these organisms can provide clues to better understand how they face environmental challenges and how to protect them. Assuming the importance of microalgae as producers in freshwater food chains, we assessed the effects in *Parachlorella* sp. of exposure (10 days) to sublethal levels of cadmium, a legacy environmental contaminant, and ciprofloxacin, an antibiotic that has been recognised as an emerging contaminant in freshwater (yield EC20 after testing according to the OECD guideline no. 201; 90.3 µg Cd/L and 7.68 mg ciprofloxacin/L). We comparatively followed total DNA methylation and several phenotypic endpoints, namely DNA damage (comet assay), physiological (growth, efficiency of photosystem II and oxygen production were assessed) and biochemical biomarkers (oxidative stress and damage). Cadmium tended to induce hypomethylation and ciprofloxacin hypermethylation of the microalgae genome. Both chemicals induced a slight decrease in biomass yield but there were no effects or slight stimulation in photosystem II efficiency and oxygen

production. Both chemicals caused mild oxidative stress and genotoxicity. Whole transcriptome analysis (RNAseq) and assessment of DNA methylation at the cytosine level (EM-seq) are ongoing, which should provide evidence on the mechanistic behind the phenotypic effects noticed and changes promoted by cadmium and ciprofloxacin in DNA methylation patterns. The molecular basis for adverse outcome pathways of toxicity caused by stressors such cadmium and ciprofloxacin in microalgae will certainly be better characterized, allowing a feasible selection of appropriate biomarkers for improved ecological risk assessment and the consequent protection of microalgae. This work was developed under the scope of project EPIBOOST, funded by the EU - Grant 101078991. AP is funded by FCT - 2022.10817.BD. JL was funded by national funds (OE), through FCT - framework contract foreseen in Decree-Law 57/2016, changed by Law 57/2017. SF was supported by FCT Individual Call to Scientific Employment Stimulus (DOI: 10.54499/2021.02653.CEECIND/CP1659/CT0012).

1.01.P-Tu002 Impact of Endocrine Disrupting Chemicals on Reproduction and Intergenerational Inheritance of Epigenetic Traces in the Freshwater Snail *Biomphalaria glabrata*

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Endocrine disrupting chemicals (EDCs) are found ubiquitously in the environment, as anthropogenic deposition has increased immensely in recent decades. They mimic or inhibit endogenous hormones and negatively impact the health of an organism through various modes of action. For example, EDCs have been shown to affect epigenetic mechanisms, which cause phenotype changes and inheritance of traits not associated with DNA sequence alteration. In this study, we investigated the effects of acute and chronic as well as direct and indirect endocrine disruption on the reproduction of the pulmonate freshwater snail *Biomphalaria glabrata* in two consecutive generations. A putative impact of estrogenic disruption in molluscs is of particular interest as this phylum has developed a distinct sexual hormone system. Nevertheless, it belongs to one of the largest classes within the animal kingdom being exposed to estrogenic substances. *Biomphalaria glabrata* individuals of the F0 generation were exposed to different concentrations of the endocrine active substance 17 β -estradiol and changes on reproduction were determined. Transcriptome analysis revealed distinct sets of differentially expressed genes in ovotestes (gonads) after a short (24 hours) and a long (28 days) exposure, while global DNA methylation was not significantly changed in this tissue. Next, long-term effects of endocrine disruption and putative intergenerational inheritance of epigenetic changes were determined. The offspring (F1) of the exposed F0 individuals were either raised without or with continuous exposure to 17 β -estradiol. Development and reproductive fitness as well as global DNA methylation of both cohorts were determined and revealed a significant negative effect of both, a direct and indirect 17 β -estradiol exposure, on the F1 generation. In summary, our data suggest that the freshwater snail *Biomphalaria glabrata* is sensitive to estrogenic disrupting chemicals and that intergenerational adverse effects could be explained by a changed DNA methylome.

1.01.P-Tu003 Multigeneration Responses of *Folsomia candida* to Short Chain Per- and Polyfluorinated Substances (PFAS)

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Due to the high persistence of per- and polyfluorinated substances (PFAS), organisms living in contaminated environments are chronically exposed to these chemicals over multiple generations. Yet, such chronic effects have barely been addressed in ecotoxicity experiments. Moreover, existing data on PFAS ecotoxicity is strongly biased towards the aquatic environment and to long-chain compounds, although short-chain PFAS have been widely used as substitutes for long-chain PFAS, resulting in high emissions into the environment. Therefore, this study aimed to assess the ecotoxicity of the short-chain PFAS perfluorobutane sulfonic acid (PFBS) and its precursor perfluorobutane sulfonamide (FBSA) to the soil invertebrate *Folsomia candida* (Collembola) over five consecutive generations, with adult survival, reproduction and population growth rate as endpoints. The tests followed OECD guideline 232, with slight modifications. In the multigeneration tests, PFBS did not significantly affect adult survival and population growth rate at the highest test concentration (1160 mg kg⁻¹ dry soil), but had a mild effect on reproduction, with EC50 values for reproduction of 700, 866 and 710 mg kg⁻¹ dry soil for the F0, F2, and F4 generation, respectively. FBSA was substantially more toxic than PFBS. Full concentration-effect relationships were obtained for all test endpoints for the F0 generations exposed to FBSA, with an LC50 of 10.3 mg kg⁻¹ dry soil, an EC50 of 1.43 mg kg⁻¹ dry soil for reproduction and an EC50 of 1.89 mg kg⁻¹ dry soil for population growth rate. Since *F. candida* exposed to 9.64 and 92.7 mg kg⁻¹ dry soil FBSA

went extinct in the F0, springtails in the following generations were exposed to FBSA concentrations up to 1.06 mg kg⁻¹ dry soil. An EC50 of 1.10 mg kg⁻¹ dry soil was obtained for reproduction in the F1 generation, but no significant adverse effect was observed on any endpoint in any other generation. It was speculated that *F. candida* could biotransform the FBSA into PFBS, but a potential biotransformation capacity threshold lies between 1.06 and 9.64 mg kg⁻¹ dry soil. It is concluded that PFBS is not very toxic to *F. candida*, but FBSA is more than 100 times more toxic, with concentration-response relationships turning into an all-or-nothing effect at a specific threshold. The comparatively high ecotoxicity of the barely tested precursor FBSA challenges the environmental risk assessment of PFAS, which is currently based on a limited number of compounds. This research was supported by the Open Technology Programme of the Dutch Research Council (NWO) [grant number 18725]

1.01.P-Tu004 Neurobehavioral Impairments in the Sea Bass (*Dicentrarchus labrax*) Chronically Exposed to Cadmium and Ciprofloxacin Contaminated Diets

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Environmental contamination by heavy metals and pharmaceuticals poses significant risks to marine ecosystems, as they can alter the behavior and survival of aquatic organisms. Cadmium (Cd) is a heavy metal recognized for its high neurotoxicity even at low concentrations such as those found in natural ecosystems. Ciprofloxacin (CIP) is a fluoroquinolone antibiotic that is increasingly detected in aquatic environments and could alter microbial communities, affecting the gut health of fish and interfering with neuronal functions through the microbiota-gut-brain axis. This study focuses on understanding the sublethal effects of these two environmental contaminants in the juvenile model of European sea bass *Dicentrarchus labrax* after 21 days of dietary exposure. For each compound, two concentrations belonging to the environmental ranges (Cd: 10 mg/kg and 100 mg/kg; CIP: 1 mg/kg and 10 mg/kg) were chosen. After exposure, we evaluated *D. labrax* behavior with a battery of behavioral tests including the social preference test (SPT), the shoaling test (ST), the light-dark test (LDT) and the group exploratory test (ET). Exposure to Cd and CIP caused significant dose-dependent behavioral alterations. In the SPT, fish exposed to higher concentrations showed less social interaction, with a reduction in the distance travelled in the conspecific compartment. In the ST, a tendency towards cohesion was observed, significant in animals exposed to the highest concentration of Cd. In the LDT, we found a pattern of anxiogenic response concomitant with depression as indicated by the prolonged time in the illuminated zone. The time in the illuminated zone increased significantly when this test was repeated as a group in the ET. Finally, we found in all cases a significant decrease in basal locomotor activity and an increase in freezing episodes.

Reduced swimming activity, changes in social behavior and alterations in exploratory and decision-making behaviors indicate that dietary exposure to Cd and CIP, even at environmentally relevant concentrations, could affect the survival and ecological fitness of the species. These results underscore the need to add sublethal endpoints in toxicological monitoring studies to preserve marine environments. Further analysis will evaluate transcriptomic and epigenetic responses and link effects at the molecular level to behavioural impairment caused by exposures to Cd and CIP.

1.01.P-Tu005 Effects of Cadmium in *Acartia tonsa*: Epigenetic and phenotypic responses

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Increasing anthropogenic activities are significantly impacting aquatic ecosystems, leading to contamination and potential deleterious effects in organisms. Cadmium, a trace metal with no essential biological role known, is particularly harmful due to its high toxicity, persistence, and bioaccumulation potential. Herein, we assessed the potential link between DNA methylation and different phenotypic endpoints as a response to short-term (48 h) cadmium exposure (238.8 µg/L, corresponding to the pre-estimated immobilization EC20) of the marine copepod *Acartia tonsa*, a zooplanktonic marine species with important ecological role and established as a model organism in ecotoxicology. Epigenetic changes were assessed through quantification of global 5-mC DNA methylation. Biomarkers of oxidative stress and damage were assessed by measuring glutathione S-transferases (GSTs) activity and the extent of lipid peroxidation, through the TBARS method, while genotoxicity was analyzed by inspecting DNA damage using the comet assay. Additionally, post-exposure swimming behavior of *A. tonsa* was analyzed by automated video-tracking. Global 5-mC DNA methylation levels slightly decreased among exposed copepods, suggesting a global hypomethylation trend. Phenotypically, this corresponded to a significant

increase in genotoxicity, a significant increase in GST activity accompanied by an increase in oxidative damage. Regarding copepods behavior, including swimming distance and swimming speed, an impairment can be noticed, but only at concentrations higher than the EC20, thus higher than those provoking recognizable effects in the other tested biomarkers. This approach supports a better understanding of cadmium's effects on marine copepods, by following adverse outcomes initiated with epigenetic mechanisms at the molecular, biochemical and individual level. As the present work becomes complete with currently ongoing whole epigenome and whole transcriptome analysis, molecular basis for adverse outcome pathways for toxicity caused by stressors such as cadmium in copepods will certainly be characterized, which should clarify further the interconnection of the responses found in the present work. Project EPIBOOST, funded by the EU through Grant 101078991; CESAM by FCT/MCTES (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020); AP by FCT - 2022.10817.BD. JL by national funds (OE), through FCT - framework contract foreseen in Decree- Law 57/2016, changed by Law 57/2017.

1.01.P-Tu006 DNA Methylation and Ocean Acidification: Insights from *Patella caerulea* at the Natural CO₂ Vent Systems of Ischia Island

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Ocean acidification (OA) represents one of the major threats to marine biota, especially to calcifying organisms. Within this context, CO₂ vent systems, which are naturally acidified sites by volcanic CO₂ emitted from the seafloor, represent a perfect window into the future, where it is possible to investigate the effects of OA on marine organisms exposed to this environmental disturbance for their entire life cycle, allowing to take into consideration any potential acclimation and/or adaptation strategies. In particular, there is evidence that epigenetic modifications, such as DNA methylation, might play a fundamental role in organisms adaptation to stress, since they can contribute to physiological plasticity but they could also promote genetic adaptation. In view of this, DNA methylation was investigated in populations of the calcifying species *Patella caerulea* collected in both winter and summer seasons from the natural pH gradient of the Castello Aragonese vent systems (Ischia Island) (N1: ambient pH = 8.1; N2: intermediate pH = 7.7; N3: very low pH = <7.4) and from the ambient pH site San Pietro (SP: ambient pH = 8.1). Genomic DNA was extracted from foot tissue of 15 individuals per site, and whole genome enzymatic methyl-seq (EMseq) libraries were constructed and sequenced on the Illumina NovaSeq 6000 sequencer. Reads were aligned to the *P. caerulea* reference genome and methylation at CpG sites was extracted using MethylKit R package. Pair-wise comparisons carried out inside the Castello Aragonese vent systems revealed that few sites were differentially methylated, indicating no difference in DNA methylation among ambient and acidified sites. Furthermore, the analysis related to seasonality (winter vs summer) within site, showed that 184 CpGs were differentially methylated in San Pietro, while inside the vent systems, the number of differentially methylated cytosines drastically reduced to 11 in N1, and to 0 in both the acidified sites N2 and N3. Previous research has highlighted that higher epigenetic plasticity might induce a greater metabolic cost to the organism. Considering that, these results could suggest that limpets living in the acidified sites of the vent may not be able to modulate DNA methylation in response to OA. Alternatively, it could be that further mechanisms are involved, such as differences in gene expression, or other epigenetic mechanisms (histone modifications, chromatin accessibility).

1.01.P-Tu007 Investigating the Effects of Glyphosate Exposure on Survival, Reproduction, and Development in *Drosophila melanogaster*

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The need for global food security has led to a reliance on chemical pesticides and their heavy use has led to severe declines in insect populations over the last 30-years. Biodiversity loss disrupts delicate ecosystems and has far-reaching consequences. Glyphosate, the active compound in widely used herbicides Gallup and Roundup, is effective, but persists in soil and water, posing acute and chronic exposure risks to humans and non-target species. Sublethal concentrations of glyphosate have been shown to have negative effects on insect development and fertility, sublethal doses reduce egg viability, alter spermatogenesis and disrupt embryonic development. This study aims to investigate the molecular mechanisms of glyphosate-based herbicide (GBH) toxicity on insect fertility and development using the fruit fly, *Drosophila melanogaster* as a model. Wild-Type Oregon-R flies are orally exposed to sublethal concentrations of GBHs. Egg development was assessed by tracking the progress of eggs laid on

contaminated medium. Fertility was determined by crossing exposed virgin flies with control virgin flies and scoring progeny over 15-days. For adults, the LC50 values for GBH Gallup were determined as 6.53mg/mL for female and 6.97mg/mL for male flies. Similarly, the LC50 for GBH Roundup was determined as 5.9mg/mL for female and 5.65mg/mL for male flies. Exposure of laid eggs to sublethal doses (0.01-5mg/mL) of Gallup and Roundup led to dose dependent declines in egg and larval survival. With 5mg/mL concentrations leading to 100% and 61% lethality respectively and 1mg/mL concentrations leading to 75% and 35% lethality respectively. Furthermore, exposure to sublethal doses of GBHs during development produced adults with impaired fertility. Females exposed to 1mg/mL Gallup or Roundup during development have 34 and 40% declines in their fertility rates. Similarly exposed males have 75 and 57% reductions in their fertility rates. Sublethal doses of GBHs have significant effects on *Drosophila* development and fertility. The formulation Gallup has a higher toxicity than that of Roundup and there are sex-specific differences in response rates to GBH exposure. Ongoing experiments aim to identify the molecular mechanisms of this reproductive toxicity. This study improves our understanding of glyphosate-induced reproductive effects and the potential effects of sublethal doses of these pesticides on insects. This Project is Currently Funded by the TUS Presidential Doctoral Scholarship.

1.01.P-Tu008 Terrestrial Ecotoxicity Evaluation of Rock Powders Used in Agriculture in Brazil: Preliminary Results with *Enchytraeus crypticus* and *Folsomia candida*

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In alignment with the principles of the Circular Economy, there is a growing expectation that waste generated in the mineral sector will not only be properly managed but also be repurposed into new production cycles. However, many materials from this sector contain naturally occurring chemicals from rocks that may pose potential toxicity. Rock powders, derived from rock extraction activities, are approved for agriculture application in Brazil as fertilizers or secondary materials. Despite this, ecotoxicological tests are not mandated under Brazilian legislation, and studies on their ecotoxicity remain limited. This lack of information is particularly concerning due to the potential long-term effects and impacts throughout the food chain. This study aims to conduct preliminary ecotoxicological tests of rock powders deemed for agriculture use, employing terrestrial organisms *Enchytraeus crypticus* and *Folsomia candida*, in accordance with ABNT standards NBR ISO 16387:2012 Soil quality Effects of pollutants on Enchytraeidae (*Enchytraeus* sp.) Determination of effects on reproduction and survival, and ABNT NBR ISO 11267:2019 Soil quality Inhibition of reproduction of *Collembola* (*Folsomia candida*) by soil pollutants. The materials were ground and sieved to ensure that at least 50% of particles were less than 0.3 mm in size. The major oxide composition included SiO₂, Al₂O₃, and Fe₂O₃. Tests were conducted by mixing the rock powder samples with Tropical Artificial Soil (TAS) at the concentrations of 0%, 0.1%, 1.0%, 10%, and 100%. Preliminary testing of three samples (SC, MG, and GE) sourced from different mining operations in Brazil (Santa Catarina, Minas Gerais and Rio de Janeiro) revealed no chronic toxicity for *E. crypticus* in any samples. However, at concentration of 100%, samples MG and GE exhibited significant chronic toxicity for *F. candida* ($p < 0.05$), with a reduction in reproduction (ANOVA followed by Dunnett's test). Complementary chemical analyses and other ecotoxicological tests need to be performed. These screening and preliminary findings highlight the importance of appropriate application rates for rock powders and integrating biological indicators into the registration process for all agricultural fertilizers and inputs in Brazil. Such measures are vital to prevent potential adverse effects on biotic communities in agricultural ecosystems while ensuring that sustainability and safety principles are upheld in the reuse of these materials. Center for Mineral Technology (CETEM/MCTI); National Council for Scientific and Technological Development (CNPq) for PCI-DA Scholarship from the Ministry of Science, Technology and Innovation (MCTI) of Brazil.

1.01.P-Tu009 Linking Molecular and Phenotypic Endpoints in Zebrafish Larvae Exposed to Ciprofloxacin and Cadmium

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The EPIBOOST project aims at validating the epigenetic modifications caused by chemicals as biomarkers of relevance which can support more accurate environmental risk analysis. For this, a multiparametric approach is pursued by looking at the effects of chemicals from the individual and biochemical levels to the genetic and epigenetic levels, establishing a link between phenotypic and molecular endpoints. In this work, taking ciprofloxacin (CIP) and cadmium (Cd) as a case study, 96 hours zebrafish larvae were exposed to sublethal concentrations of the CIP (ranging from 10 to 50 mg/L) and Cd

(0.24 to 24 μ g/L) for 24 hours. Endpoints analysed included mortality, development, swimming behaviour (swimming distance and path angles), biotransformation enzymes (glutathione-s-transferase (GST)), antioxidant enzymes (catalase (CAT), and glutathione reductase (GR)), neurotoxicity markers (acetylcholinesterase (ChE)), transcriptomics and DNA methylation. For Both chemicals, at concentrations tested no effects on survival or development (delays or anomalies) were observed. CIP induced an activation of the antioxidant system which seemed to have occurred as suggested by the increase in the CAT activity. In contrast to Cd which decreased the CAT activity. In the same direction, an increase in ChE activity was also observed in eleutheroembryos exposed to CIP, while Cd induced a reduction in the ChE activity suggesting neurotoxicity elicited by the tested Cd concentration. The study of swimming behaviour indicated an alteration of the swimming pattern, where zigzag movements and high-speed movements increased suggesting an anxiety-like behaviour of larvae exposed to the antibiotic. Molecular analyses are underway to further elucidate transcriptomic changes and DNA methylation profiles associated with these phenotypic and biochemical alterations.

1.01.P-Tu010 Effects of Bisphenol S (BPS) in the Threespine Stickleback (*Gasterosteus aculeatus*): Exploring the Links Between Biomarker and Life History Traits Responses

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Chemical pollutants in aquatic environments disrupt biomarkers, affecting growth, reproduction, and survival. Bisphenol S (BPS), a substitute for bisphenol A (BPA), has been linked to reproductive impairments, metabolic disruptions, oxidative stress, and immune dysfunction in fish. This study investigates BPS effects on biomarkers and life-history traits in three-spined stickleback (*Gasterosteus aculeatus*) to establish mechanistic links across biological scales. Three experiments were conducted: (1) a 6-month mesocosm study exposing fish to BPS (0, 1, 10, 54 μ g/L) in artificial streams, assessing biomarkers like immune parameters, respiratory burst, and organ mass; (2) a 21-day caging study in mesocosms examining reproductive and oxidative stress biomarkers; and (3) a 40-day laboratory study (BPS: 0, 1, 10, 100 μ g/L) assessing biomarkers and linking them to life-history traits such as stickleback nest construction in males and oocyte maturation in females. The mesocosm experiment revealed altered respiratory burst, reduced immune cell counts, and impaired phagocytic function in both sexes, suggesting immunosuppression, with females also exhibiting cells necrosis. In the 21-day study, both sexes showed signs of altered antioxidants, with females showing effects at low doses (1 μ g/L). Laboratory experiments revealed significant effects on reproduction, metabolism, and links between biomarkers and life history traits. Dose-dependent effects included reduced gonado-somatic index (GSI) and sperm count in males, alongside increased nephrotic-somatic index (NSI) and variability in nest characteristics (weight, surface area). In females, hepatosomatic index (HSI) increased significantly, while mature oocyte count and GSI increased at higher doses. The study revealed effects of BPS on reproduction, metabolism, oxidative stress, respiratory burst and phagocytic activity, and allow to establish link biomarkers to life-history traits. This approach aims to model population-level impacts and extrapolate findings to other pollutants, enhancing environmental risk assessment. This project has also received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement No 101057014 (The European Partnership for the Assessment of Risks from Chemicals; PARC).

1.01.P-Tu011 Embryonic Exposure to the Endocrine Disrupting Chemical, TBCO, Causes an Intrageneration Decrease in Reproductive Performance of Female Japanese medaka (*Oryzias latipes*) by Impairing Oocyte Maturation

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Vitellogenesis and oocyte maturation are the final two steps of oogenesis in fish. Most studies of reproductive toxicity focus on the disruption of vitellogenesis leading to decreased fecundity, with very little focus on oocyte maturation, the final stage of oogenesis that gives rise to a fertilizable oocyte. Previous studies from our group demonstrated that reduced fecundity of Japanese medaka exposed as embryos to maternally transferred TBCO, a brominated flame retardant, was caused by impairment of maturation-inducing hormone (MIH)-induced oocyte maturation. However, the molecular mechanism(s) of this effect are unknown. The objective of this research is to investigate the molecular basis of decreased oocyte maturation leading to reduced fecundity in Japanese medaka exposed as embryos to maternally deposited TBCO. F0 generation fish were fed a 100 or 1000 μ gTBCO/g diet for 21 days. F1 generation embryos collected during the final week of the exposure were reared to sexual maturity in clean water without any additional exposure to TBCO. At sexual maturity, reproductive performance was assessed in a standard 21-day reproduction assay, after which an ex-vivo oocyte maturation assay was performed. To elucidate mechanisms of decreased oocyte maturation, high-throughput RNA sequencing and enzyme-

mediated methyl sequencing were used to compare the transcriptome and methylome of the oocytes that failed to mature and those that matured from control and TBCO-exposed female fish. Exposure to a maternally transferred TBCO caused a concentration-dependent decrease in fecundity and oocyte maturation. In progeny of fish given the 1000 ugTBCO/g diet, the decrease in fecundity was 31.4% and the decrease in oocyte maturation was 22.3%. Transcriptomics analysis showed that genes responsible for regulating mitochondrial energy generation and non-genomic signal transduction were altered in oocytes that failed to mature. Enzyme-methylated sequencing is being used to determine if changes in DNA methylation might alter the gene expression that regulates oocyte maturation. This research will advance the understanding of how early life-stage exposure to environmental chemicals can impact the reproductive performance of fish. This research was supported by a Tier II Canada Research Chair in Aquatic and Mechanistic Toxicology, and a Discovery Grant from the Natural Science and Engineering Research Council (NSERC) of Canada, to Steve Wiseman.

1.01.P-Tu012 Two-Generation Toxicity Assessment of the Antioxidant Propyl-Propane Thiosulfonate (PTSO)

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Propyl-propane thiosulfonate (PTSO), an antioxidant organosulfur compound found in the *Allium* genus, shows promise as a natural additive for food and feed, as well as a biopesticide for plant pest control. To ensure its safety for livestock, consumers, and the environment, a comprehensive toxicological assessment is essential. This study aimed to evaluate the potential reproductive toxicity of PTSO in mice, following OECD guideline 416 as part of a broader risk assessment. Spanning two generations, the study assessed possible reproductive, teratogenic, and genetic impacts. A total of 80 CD1 mice per sex per generation were exposed to PTSO through feed at three dose levels (14, 28, and 55 mg PTSO/kg body weight/day) across pre-mating, gestation, and lactation phases. The study observed no clinical changes or mortality linked to PTSO exposure. While some variations in body weight and food intake occurred, they were neither dose-dependent nor sex-specific. Both parental generations (F0 and F1) exhibited normal reproductive outcomes, and offspring (F1 and F2) were born without abnormalities. Sexual hormone levels (progesterone, testosterone, estradiol, follicle-stimulating hormone, and luteinizing hormone) remained within normal ranges. Although sperm analysis showed significant changes in the F0 group, no such variations were observed in F1, and no fertility impairments were noted. Additionally, organ weights (absolute and relative) and detailed histopathological examinations revealed no significant differences across both genders and generations. In conclusion, under the tested conditions, PTSO does not exhibit reproductive or developmental toxicity in mice, supporting its favorable safety profile for potential use in the agrifood industry. This research was funded by Junta de Andalucía (Project P18-TP-2147), and by the Spanish Ministerio de Universidades through the FPU grant (FPU2019-01247) awarded to Antonio Cascajosa Lira.

1.02.P The Chemical Defensome: Novel Insights into the Mechanisms of Defense Allowing Species to Cope with Environmental Pollution

1.02.P-Tu013 Evolution of the Chemical Defensome in Marine Mammals

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Many marine mammals such as whales, seals, and polar bears, are top-predators, feeding high in the food chain, resulting in a high intake of persistent, bioaccumulating pollutants with the potential to cause harm to the organism. The chemical defensome comprises an integrated network of gene families and pathways that together function to sense, metabolize and eliminate harmful compounds. Although highly conserved, notable evolutionary adaptations are observed through mammalian evolution, most notably in cetaceans. The absence of functional nuclear receptors PXR and CAR in cetaceans, and their presence in polar bears, pinnipeds and sirenians, make the case for an interesting comparison of how these different marine mammal species sense and respond to environmental toxicants targeting these receptors. Our main hypothesis is

that the genomes of marine mammals can be decoded into a functional understanding of the responses and susceptibility to toxicants of these animals. Publicly available genomes (NCBI/Ensembl) were used for genome mining, targeting the genes representing the chemical defensome of 40 selected marine mammal species and 28 close relatives. A specific focus is on defensome gene complements, functional gene networks, phylogenetic relationships, gene gain/loss and implications for evolutionary adaptations. The functional defensome gene inventory was then compared between marine mammal lineages and their close relatives. Our analysis revealed large scale loss of defensome genes in cetaceans compared to closely related species. A total of 101 defensome genes were estimated to be lost compared to the reference gene sets. Gene losses are found across the whole spectrum of defensome genes, ranging from transcription factors (NR1I2 and NR1I3), oxygenases (CYP2 family) and reductases (NQO1), to transferases (UGT, GST) and transporters (ABC, OATP/SLCO). Marine mammals that have not lost PXR and CAR (pinnipeds and sirenians), do not show these wide-spread defensome losses. However, both lineages show losses in oxygenases (CYP2 family and FMO) and transferases (GST, SULT). Overall, our results suggest a significant remodelling of the chemical defensome in cetacea. Sporadic gene loss events were also observed in pinnipeds and sirenians. The obtained dataset will serve as a genomic framework to comparatively address the convergent and divergent susceptibilities towards chemical pollution across marine mammals. The authors thank the Research Council of Norway for funding the Marma-detox project (Whales and polar bear in a petri dish: decoding marine mammal toxicology through in vitro and in silico approaches, RCN project #334739).

1.02.P-Tu014 Exploring the Role of the Defensome in Endocrine Disruption: Interspecies Differences in Sensitivity to Thyroid System Interference

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Thyroid homeostasis can be affected by increased hepatic clearance of circulating thyroid hormones, in response to upregulation of phase I and II metabolising enzymes. Induction of these enzymes is often mediated by xeno-sensor receptors e.g. CAR/PXR, but there appears to be variation among taxa in the presence, role and specificity of these receptors. In the context of EU regulations, forbidding registration of pesticidal active substances with endocrine disrupting (ED) properties, the status of this mode of action for thyroid disruption is ambiguous. The criteria for identifying EDs state that adverse effects that are non-specific secondary consequences of other toxic effects shall not be considered ... We interrogated the database of substances subject to Tier 1 determinations under the US EPA Endocrine Disruptor Screening Programme (EDSP), for evidence that liver-mediated effects on the thyroid system in human health models correlate with thyroid activity in the amphibian metamorphosis assay (AMA). We categorised AMA endpoints, and effect patterns indicative of thyroid activity were established based on responses to known thyroid disruptors in the OECD validation of the AMA test guideline. These responses were then compared to thyroid responses in male and female pubertal assays, and responses of in vitro assays for CAR/PXR activation conducted under the US EPA ToxCast program. Among the 39 substances assessed, it was concluded that 13 affected the thyroid axis: 2 affected amphibians only, while 9 only affected mammals. Another 2 affected amphibians and mammals, though it should be noted that one of those did not exhibit a clearly thyroidal response pattern in the AMA. Among the 9 affecting thyroid parameters in mammals only, 6 did so indirectly, secondary to induction of liver enzymes. None of those substances were negative in ToxCast screens for CAR/PXR activation. None of those 6 substances elicited thyroid effects in the AMA. These data indicate that the AMA model is rather insensitive to perturbations of the thyroid axis consequent to CAR/PXR-mediated liver enzyme induction. This suggests that, in relation to thyroid disruption, read-across from amphibians to mammals will be less protective than the converse, if liver-mediated thyroid insufficiency should be regarded as a specific effect. This assumes greater importance given current interest in developing new approach methodologies to support ED identification while reducing animal use.

1.02.P-Tu015 Biochemical Responses of *Danio rerio* and *Daphnia magna* to pharmaceuticals Isoeugenol and Altrenogest

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Industrial discharges, intensive agriculture, and improper waste disposal contribute to the presence of harmful chemicals, including pharmaceuticals, in aquatic environments. These substances, originally designed for specific purposes, can interfere with physiological and biochemical processes in non-target organisms. This study investigates the effects of two pharmaceuticals identified as priority substances by the European Medicines Agency, Isoeugenol (ISO) and Altrenogest (ALT), on the aquatic species *Danio rerio* and *Daphnia magna*, by assessing alterations in enzymatic biomarkers. Enzymatic biomarkers are early indicators of physiological stress and biochemical disturbances in organisms exposed to pollutants.

ISO, a natural organic compound known for its anti-infective properties, and ALT, a synthetic progestin used in veterinary medicine, represent two pharmaceuticals with differing mechanisms of action, enabling a comparative analysis of their ecotoxicological impacts. The concentration range for the tests was based on previous acute toxicity data, from which LC50 values were derived for both species. For *D. rerio*, concentrations of 0.09 to 1 mg/L were used for ALT, and 0.37 to 7 mg/L for ISO, with a 120-hour exposure period. For *D. magna*, the concentration ranges were 0.04 to 0.27 mg/L for ALT and 0.14 to 2.62 mg/L for ISO, with a 21-day exposure period. A battery of biomarkers was selected for a comprehensive assessment of the ecotoxicological effects. From the antioxidant defence mechanism, Catalase (CAT), Glutathione Peroxidase (GPx), and Glutathione Reductase (GR) were chosen to evaluate the organism's ability to combat oxidative stress. Additionally, Glutathione S-Transferase (GST), a key enzyme in phase II biotransformation, was selected to assess detoxification processes. Lipid Peroxidation (LPO) was measured to evaluate oxidative damage to cellular membranes. Exposure to ISO and ALT caused significant oxidative stress in the tested organisms, as indicated by alterations in the activities of CAT, GPx, GR and GST. The changes in these key antioxidant and detoxification enzymes suggest a weakened ability to neutralize free radicals and process toxins, compromising cellular defences. Increased LPO further suggests damage to cell membranes, highlighting the potential of both compounds to cause cellular damage and disrupt biological functions, particularly in antioxidant systems and cellular integrity.

1.02.P-Tu016 Defense Mechanisms of Sulforaphane Against Environmental Toxins and Contaminants: A Systematic Review

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Sulforaphane (SFN) is a biologically active isothiocyanate found in cruciferous vegetables, particularly those from the Brassica genus, and is derived from the precursor compound glucoraphanin. Known for its numerous health benefits, SFN acts primarily as an inducer of nuclear factor erythroid 2-related factor 2 (Nrf2), a key regulator of antioxidant response. As a result, SFN has demonstrated a variety of protective effects, including anticancer properties and the ability to shield against toxic agents. This systematic review aimed to examine and synthesize the existing evidence on SFN's protective role, particularly in relation to toxic substances and contaminants, and to explore the underlying mechanisms through which it exerts these effects in various organs and systems. The review found that SFN's protective activities are most pronounced in the liver and nervous system, both of which are highly susceptible to damage from oxidative stress, a hallmark of many liver and neurodegenerative diseases. However, its protective effects are not limited to these organs; SFN has also been shown to benefit other systems, such as the lungs, heart, kidneys, immune system, and endocrine system. The mechanism by which SFN exerts these effects involves the activation of the Nrf2 pathway, which enhances the body's antioxidant defenses, reduces oxidative stress, and modulates the expression of protective genes. Additionally, SFN has been shown to reduce inflammation by decreasing the production of pro-inflammatory cytokines like interleukins and to inhibit apoptosis by preventing caspase-3 activation while increasing the levels of anti-apoptotic proteins like Bcl-2. These multifaceted mechanisms suggest that SFN can effectively counteract the detrimental effects of various toxic agents. Despite its promising potential as a chemoprotective agent, further studies are needed to establish safe and effective dosages for human use and to better understand its long-term effects in clinical settings. This research was funded by Junta de Andalucía (Project P18-TP-2147), and by the Spanish Ministerio de Universidades through the FPU grant (FPU2019-01247) awarded to Antonio Cascajosa Lira.

1.02.P-Tu017 Polymorphisms of the Metallothionein 2A Gene in Relation to Heavy Metal Levels in a Colombian Population

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Genetic variations can be considered as internal factors contributing to the susceptibility of individuals to heavy metal-related toxicities. Metallothioneins are highly conserved, cysteine-rich, low molecular weight metal-binding proteins with multiple cellular functions. Metallothioneins are involved in metal homeostasis and transport and have antioxidant and anti-inflammatory properties. Their synthesis can be induced by essential elements such as Zn and Cu, heavy metals, chemical agents and stress-producing conditions. MTs suppress heavy metal toxicity by binding to these metals. 24 SNPs have been identified in the MT2A gene and three of them have been shown to be associated with metal concentrations in the body of healthy and diseased individuals. This study aimed to investigate the association of single nucleotide polymorphisms rs28366003, rs1610216 and rs10636 of the Metallothionein 2A (MT2A) gene and arsenic, cadmium, mercury and lead levels in blood samples from 95 Colombian adult subjects. 95 healthy subjects from the department of Bolivar were enrolled. Whole blood samples were taken to quantify the

levels of four elements (arsenic, cadmium, mercury and lead) by ICP-MS. TaqMan® probes were used for polymorphism analysis in real-time PCR systems. Single nucleotide polymorphisms (SNPs) were genotyped in blood samples using TaqMan allelic discrimination assays. The genotype distribution of MT2A rs28366003, rs1610216 and rs10636 conforms to Hardy-Weinberg equilibrium. Statistically significant associations were detected between metallothionein 2A (MT2A) SNPs and metal and metalloid levels in the study population. These results suggest that MT2A rs28366003, rs1610216 and rs10636 polymorphisms increased the risk for adverse health effects from heavy metal exposure in the Colombian population.

1.02.P-Tu018 Effects of Oxyfluorfen on Biochemical Activities in Non-Target Aquatic Invertebrate Organisms

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Herbicides are chemical substances used to control weeds in agricultural fields. Oxyfluorfen (OXY) is herbicide used in agricultural processes of crops such as soybeans, rice fields, and peanuts. Freshwater pollution from agricultural activities causes various effects on aquatic organisms. The present study evaluated the effect of acute exposure of OXY on non-target aquatic invertebrate. For this purpose, freshwater mussels (*Unio delicatus*) were divided into 7 groups and exposed to OXY at concentrations of 500, 1000, 1500, 2000, and 2500 µg/L for 96 hours. There were negative and solvent control groups. After weight and shell length measurements (mean weight of 40.1 ± 6.9 g and mean shell length of 66.9 ± 3.5 mm), gill and digestive gland samples were taken from the mussels. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), and malondialdehyde (MDA) antioxidant parameters were investigated. Although no lethal effects were observed in mussels exposed to OXY, biochemical effects were observed. The antioxidant defense system showed significant activation of SOD and MDA in mussels exposed to OXY ($p < 0.05$). On the other hand, there was no significant change in CAT, GPx and GSH levels in OXY exposure. Integrated Biomarker Response (IBRv2) was obtained lower in the digestive gland than in the gill tissue. The increase in SOD and MDA in the gill and digestive gland tissues of mussels exposed to OXY indicates that oxidative stress has begun in the organism and that superoxide radicals and lipid peroxidation are at the forefront. On the other hand, the fact that GPx, CAT and GSH did not show any change may suggest that the antioxidant defense mechanisms of the mussels give a limited or delayed response. Higher IBRv2 in gill tissue indicates that the organism is facing more intense stress in this tissue and that defense mechanisms are more activated. This may be related to direct exposure of the gills to environmental toxicants. Lower IBRv2 in the digestive gland may indicate that this tissue is less exposed to environmental stress or is able to cope more effectively. As a result, it was revealed that OXY herbicide caused oxidative stress in freshwater mussels in all applied dose ranges. The study was funded by Gazi University Scientific Research Unit (Project number: SPD-2024-9697). Gulsum Batmaz Erismis was supported by TUBITAK 2211 -A Domestic Doctoral Scholarship Program 2022/1.

1.02.P-Tu019 The Evaluation of the Effect of Sublethal Diflubenzuron on Antioxidant Enzyme Systems in Freshwater Mussels

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The use of pesticides in agriculture, domestic and municipal areas is increasing day by day. Diflubenzuron (DFB) is an insecticide used as an insect growth regulator in agricultural applications and is used especially in cotton, apple and pear cultivation. Additionally, in some developed countries it is used on mosquito larvae to prevent diseases spread by mosquitoes. In this way, intensive use harms ecosystems and populations in these ecosystems. These chemicals mixed into aquatic ecosystems cause undesirable effects on aquatic organisms. In the current study, we evaluated the effects of DFB, one of the common insecticides, on antioxidant enzyme systems of freshwater mussels. The freshwater mussels (*Unio delicatus*) were divided into control and sublethal DFB-exposed (1.24 mg/L and 12.4 mg/L) groups for 48 hours and 7 days. After measuring the weight and shell length of the mussels (mean weight 32.91 ± 1.29 g and mean shell length 4.94 ± 0.11 mm), digestive gland and gill tissues were collected from the mussels and antioxidant enzyme biochemical parameters were examined. Inhibition of superoxide dismutase activity in digestive gland and gill tissues was observed in long-term exposure to sublethal DFB concentrations. On the other hand, an increase in glutathione peroxidase activity was observed in digestive gland tissue during both exposure periods of mussels to DFB concentrations. There was no change in tissue catalase activity in both exposure times and DFB concentrations. The findings suggest that DFB has a toxic effect on mussels, leading to changes in tissue antioxidant enzyme parameters. The study provides

information on environmental risks associated with pesticides such as DFB, highlighting the importance of using freshwater mussels as an alternative model organism for toxicity assessments. The study was funded by Gazi University Scientific Research Unit (Project number: FYL-2022-7938).

1.02.P-Tu020 Surviving in the Sediment: Behavioral and Toxicological Effects of Nano-biocides on the Marine Gastropod *Gibbula umbilicalis*

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Antifouling (AF) biocides, such as DCOIT, and corrosion inhibitors with biocidal activity, such as MBT, are widely used to prevent (micro)biofouling but pose significant risks to non-target marine organisms. Their nanoencapsulation in silica nanocapsules (SiNC), with or without silver impregnation (SiNC-Ag) for dual biocidal effect (SiNC-Ag-DCOIT), has been proposed to control the biocides release over time, reducing their toxicity while maintaining the AF effectiveness. However, the toxicity of such novel nanoadditives toward marine species is still not thoroughly investigated, particularly in benthic species. Therefore, the present study evaluates the acute toxicity (48 h) of DCOIT and MBT, both in their soluble and nanostructured forms, using the marine gastropod *Gibbula umbilicalis* as a model organism that plays a crucial ecological role in nutrient cycling and sediment stabilization in saltmarshes. Sediment toxicity tests were conducted at concentrations of 1, 10, and 100 mg/kg (n=3, five gastropods per replicate). Avoidance of contaminated sediment and mortality were assessed to determine the No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) and to compare the toxic effects of tested chemicals. The results indicated that SiNC alone was non-toxic, with mortality rates of up to 13% at the highest concentration tested (100 mg/kg). DCOIT exhibited high toxicity with a LOEC = 1 mg/kg for both behavioral and mortality endpoints. Encapsulation of DCOIT in SiNC and SiNC-Ag partially reduced toxicity at lower concentrations, but failed to prevent 100% mortality at 100 mg/kg. SiNC-DCOIT and SiNC-Ag-DCOIT caused similar lethal effects, indicating limited effects linked to the silver coating. In contrast, soluble MBT was more toxic than DCOIT, with a mortality of 80% as low as 1 mg/kg (LOEC) and 93 100% at higher concentrations. Encapsulation (SiNC-MBT) failed to mitigate MBT toxicity. These findings highlight that MBT is more toxic than DCOIT to *Gibbula umbilicalis*. These findings underscore the need for further optimization of the encapsulation strategies. This study advances our understanding of the environmental risks posed by biocides and emphasizes the importance of developing tailored nanoencapsulation approaches for safer and more environmentally sustainable antifouling technologies. This research was funded by the São Paulo Research Foundation (FAPESP), grant number 2024/01138-0. We acknowledge the financial support of the Compete 2030/FEDER to the NANOBIOESCUDO project (COMPETE2030-FEDER-01194000) and from the Portuguese Foundation for Science and Technology (FCT) to the project NANOGREEN (CIRCNA/BRB/0291/2019; DOI: 10.54499/CIRCNA/BRB/0291/2019), to CESAM (UIDB/50017/2020+UIDP/50017/2020+LA/P/0094/2020) and to R. Martins (2021.00386.CEECIND; DOI: 10.54499/2021.00386.CEECIND/CP1659/CT0011).

1.03.P Developing Science-Based Metrics to Quantify Fashion and Apparel's Chemical and Biodiversity Impacts on Nature

1.03.P-Tu021 Textile Case Study for Integrated Chemicals and Waste Management

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This paper presents a case study from the textile sector on economic and industry sector engagement and sectorial actions, addressing climate change, biodiversity loss and pollution. It is put into context of a discussion note entitled Development of Global Framework on Chemicals (GFC) implementation programmes to advance integrated chemicals and waste management: National systems, industry engagement, and sustainable development linkages. The textile sector is as one of the key economic and industry sectors for fostering early engagement and for creating momentum. It has strong impacts on all three dimensions of the triple planetary crisis, making it a prime target for integrated action. ISC3 builds on multi-stakeholder cooperation with textile initiatives and organisations such as the ZDHC Foundation who themselves are working on textile solutions in a multi-stakeholder approach. A key output of the multistakeholder consultation and interactions has been the ZDHC Manufacturing Restricted Substances List (MRSL), a list of chemicals banned from intentional use in the processing of textile materials, leather, rubber, foam, adhesives and trims used in textiles, apparel, and footwear industry. ISC3 in a collaboration with ZDHC aims at strengthening outreach and capacity building, and finally have started working on a concept supporting the transfer of the MRSL to other sectors such as consumer electronics. In relation to promoting innovative solutions of sustainable chemistry in the textile sector, the ISC3 Innovation Hub is

supporting start-ups working on innovative sustainable chemistry solutions for the textile sector around the globe. The identified innovative examples have a high potential for transforming distinct steps in the textile value chain. Combining and integrating key innovations developed by different start-ups and large corporates along the textile value chain can and will further enhance the impact of these more sustainable solutions. It is therefore of high importance to facilitate these connections and to build-up business partnerships. As one step towards this goal, ISC3 is currently facilitating a corporate challenge with a frontrunner in sustainable textile and fabrics manufacturing and supply to find start-ups offering innovative low-carbon bio-based and less hazardous alternatives to the traditional textile chemicals.

1.03.P-Tu022 Development of a Rapid Screening Method for Detection of Hazardous Additives in Textiles

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Flame retardants (FRs), per- and polyfluoroalkyl substances (PFAS), antimicrobials, and dyes are intentionally added to textiles to provide protection from fire, stain/water resistance, odor control, and color. However, these additives pose a number of health concerns, including reproductive health risks, skin irritation, and increased risk of cancer. When these classes of chemicals are added to textiles used in uniforms that must be worn regularly, such as those of first responders, school children, flight attendants, postal workers, military personnel, food service and hospitality workers, they represent a public health or occupational health threat that is largely unstudied in the United States. Traditional analyses for the presence of PFAS, metals, and flame retardants in textiles typically require separate, specific extraction protocols and analysis using multiple instruments. In an effort to save analysis time and resources, the current work has developed a rapid screening methodology to identify hazardous additives in textiles, and applied the methodology to a variety of samples, including firefighter station wear. First, samples underwent the ex-vacuo techniques of particle-induced gamma-ray emission (PIGE) and particle-induced x-ray emission (PIXE) spectroscopy to perform nondestructive elemental analysis. In three minutes per sample, textiles were screened for F, Cl, Br, Cr, Zn, and Cu as a surrogate for the presence of PFAS, flame retardants, toxic dyes, or antimicrobials. Due to the presence of Br or Cl being from a FR or dye, samples shown to contain these elements then underwent a secondary thin layer chromatography screening method to differentiate dyes from FRs. A subset of samples then underwent the following analyses to validate the screening methods: targeted LC-MS/MS analysis for PFAS, GC-MS analysis for FRs, HRMS for brominated/chlorinated dyes, and ICP-OES to confirm presence of metals. Preliminary results confirm a variety of concerning chemicals can be identified rapidly in firefighter station wear and other textiles.

1.03.P-Tu023 Impact of Microfibre Emissions from Textile Industries

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Dyed cellulosic fibres (natural and regenerated) account for 80% of microfibrils found in seafloors, raising concerns over their impacts on the environment. Most research into microfibrils concentrates on laundry and identifies it as a major cause of microfibre leakage into the environment; however, this typically ignores microfibre shedding during the various stages of textile manufacturing. Microfibrils are typically <5 mm, which increases their potential to enter the food chain where they are consumed and entangle as clots, causing blockages within the gut of aquatic organisms. Fibres in the form that they occur in plants may not be harmful to flora and fauna, but the use of textile auxiliaries, strong alkali, detergents, oxidising agents, dyes, and finishes during textile processing changes the cellulose polymer crystal structure and chemistry, and covalently bonds compounds to the fibres, all of which permanently changes fibre structure and chemistry from that observed in nature. Attached chemistry also has the propensity to be released into the environment during degradation, the effects of which are unknown. Therefore, mitigation strategies at every step of textile manufacturing should be implemented to reduce microfibre emissions. From our study, during the dry process- the use of ring, compact, and air-jet spinning have potential to release less microfibrils during fibre spinning. For knitting and weaving- laser cut rib filament knit, and unprocessed twill filament or plain spun weave would shed less microfibrils. During dyeing, the use of dope dyeing, supercritical CO₂ dyeing, and cold pad-batch dyeing have potential for positive impact on the environment to reduce microfibre emissions. Effluent treatment using combination of membrane bioreactor and biological catalysis, or ultrafiltration with crossflow module and reverse osmosis, or acoustic technology are improved industrial wastewater treatment practices that could be used to prevent the unintended release of microfibrils. The microfibrils recovered from the textile effluents can further have applications in fibre-to-fibre recycling, fillers in bio-composites, or 3-D printing. To conclude, implementing better-by-design interventions, using economically viable and scalable models for industries at manufacturing stage along with increasing consumer awareness will help tackle the microfibre

challenges. This work was funded by the National Environment Research Council (NERC) as listed by UKRI, Future Fibres Networking Grant, NE/Y003985/1.

1.03.P-Tu024 Forest Biodiversity Indicators for Sustainability Reporting in the Wood-Based (Regenerated Cellulose) Fiber Industry for Fashion

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Most studies on assessing the environmental sustainability of apparel are primarily focused on pressure indicators like air emission, water pollution or land use. However, to assess biodiversity, state indicators are also required, and it is advisable to collect information on these at the site of the ecosystem intervention. The wood fiber-based fashion industry heavily depends on wood as a raw material, which poses potential risks to forest biodiversity. As sustainability becomes increasingly important, companies are applying science-based environmental targets, driven by regulations such as the Corporate Sustainability Reporting Directive (CSRD). Among other topics, this regulation requires companies to disclose their impacts and dependencies on biodiversity. However, currently available biodiversity indicator sets tend to be generic and only deal superficially with different economic sectors. Science-based indicators and goals can help companies make informed biodiversity commitments. Collaboration among stakeholders, including regulatory experts, researchers, and businesses, is essential to align corporate actions with biodiversity goals. We therefore want to utilise results of forest biodiversity research for practical application in corporate sustainability reporting. This study uses a Delphi survey to identify and evaluate forest biodiversity indicators suitable for corporate sustainability reporting in the wood fiber-based fashion industry. The indicators should be used to assess the impact on biodiversity at the point of wood procurement. Experts from the fields of sustainability regulatory and biodiversity research are consulted to propose relevant indicators and assess their practical application. The goal is to provide businesses with knowledge to better integrate biodiversity considerations into their sustainability strategies. Initial findings suggest that while meaningful indicators are available, access to timely and spatially accurate data remains a challenge. Further research is needed to develop sector-specific indicators that meet the requirements of sustainability reporting.

1.03.P-Tu025 Embryonic Exposure to Alizarin Yellow Dye Alter Embryonic Development and Behavior in Zebrafish (*Danio rerio*)

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In recent years the use of synthetic dyes has increased significantly, however, little is known about their ecotoxicological effects. Among different classes of dyes, the azo dyes are used in great quantities and are known to break down into carcinogenic amines. One amine of interest is alizarin yellow. This dye is water soluble and has various applications, including in the coloring of textiles, leather, and plastics and the manufacture of paints and lacquers. Given the widespread use of this dye, it has been considered a major contaminant of aquatic environments, and bodies of water contaminated with this dye are some of the most difficult to treat due to the complex aromatic structure of the dyes, which makes them more stable and difficult to biodegrade. However, little is known about the toxicity of alizarin, including toxicity to early life stages of fish. In this study, freshly fertilized zebrafish embryos were microinjected with either a DMSO control or with 38, 190, 950, 4750 ng/g egg of alizarin yellow before gastrulation, and effects on survival, hatching success, and malformations were assessed until 14 days post-fertilization. Heart rate was quantified at 48 hours post-fertilization, and a behavioral assay to quantify locomotion was performed on larvae at 5 days post-fertilization. Alizarin yellow caused dose-dependent increases in mortality, with an EC50 of 3129 ng/g egg. There was a significant decrease in mean heart rate in the highest treatment (178 beats per minute) compared to the control (170 beats per minute) at 48 hours post-fertilization. Dose-dependent increases in the occurrences of malformations were observed, including a significant reduction in the length of the larva, a significant decrease in the eye area, curvature of the spine, pericardial edema, and yolk sac edema were also observed. The occurrence of each malformation was significantly higher at the highest dose of alizarin yellow. Overall, embryonic exposure of zebrafish to alizarin yellow dye affected the embryonic development of zebrafish which may have ecotoxicological implications. Additional research is being performed to understand the mechanisms of toxicity and the risk that alizarin yellow may pose to aquatic organisms.

1.03.P-Tu026 Measuring So We Can Manage: The Current Landscape of Textile Chemical Impacts on Biodiversity and Ecological Health

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The interconnected crises of climate change, pollution, and biodiversity loss, as highlighted by the United Nations Environment Programme (UNEP), underscore the urgency of addressing chemical pollution. Within the textile and apparel industry, harmful chemical emissions significantly impact biodiversity and water ecosystems, yet data and methodologies to measure these impacts remain insufficient. This research examines the current landscape of chemical pollution in the textile supply chain, focusing on its ecological consequences and the gaps in data, tools, and methodologies. A comprehensive review of scientific literature, industry reports, and non-governmental organization (NGO) assessments identified critical challenges and gaps, particularly the lack of standardized metrics connecting chemical emissions to biodiversity outcomes. Current tools, such as geographical risk tools, provide valuable insights but lack the granularity needed to inform mitigation strategies specific to textile industry pollutants. Collaboration between chemical and ecological disciplines is essential to bridge these gaps. This study highlights the necessity for developing practical, scientifically robust indicators to track chemical footprints in line with emerging disclosure frameworks such as the EU Corporate Sustainability Reporting Directive (CSRD). By documenting ongoing initiatives and fostering cross-sector collaboration, efforts like the Zero Discharge of Hazardous Chemicals (ZDHC) program aim to operationalize solutions, reduce research redundancy, and enhance resource allocation. The findings underscore the need to establish reliable impact metrics and frameworks that not only address immediate challenges but also support long-term goals for safer chemical practices and sustainable biodiversity management. Documenting existing efforts will reduce redundancy, encourage cross-sector collaboration, and optimize resource use for future research.

1.04.A Molecular Ecotoxicology and Omics Perspectives: Advancing Mechanistic Understanding for Environmental Risk Assessment

1.04.A.T-01 Exploring Mechanisms of Chemical Toxicity Using *Chlamydomonas reinhardtii* Mutants Library

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The molecular mechanisms underlying chemical toxicity and stress response are often poorly understood. Functional genomics, which links genetic determinants to phenotypic outcomes, provides a high-throughput approach to uncover genes and pathways involved in chemical susceptibility and tolerance. Using a library of ~11,000 *Chlamydomonas reinhardtii* gene knockout mutants, we investigated responses to 15 chemicals, including herbicides, heavy metals, and a cyanotoxin. By identifying mutants with altered growth under chemical exposure, we aimed to elucidate key molecular mechanisms of toxicity. Following three days of exposure to chemicals, DNA barcodes identifying each mutant were sequenced and analyzed. Logarithmic fold change of growth was calculated and gene set enrichment analyses were performed using various functional gene sets. Across treatments, mutations more often increased susceptibility than tolerance. The number of tolerant mutants across treatment ranged from 2 (microcystin) to 192 (glyphosate), while susceptible mutants ranged from 10 (atrazine) to 2,076 (silver). Some genes consistently influenced responses across treatments; e.g., Cre06.g278116 conferred tolerance in 10 treatments, while Cre17.g741150 caused susceptibility in 14. Tolerance was not enriched for specific processes, but susceptibility was associated with genes involved in mating and early zygote formation. Certain mutants exhibited opposing responses depending on the treatment. For instance, Cre17.g722150 (a Type III polyketide synthase) conferred susceptibility to seven chemicals but tolerance to six, while Cre17.g702150 (a thioredoxin-like protein) mostly mediated resistance to metals but susceptibility to herbicides and microcystin. Chemical-specific responses were also observed, such as cation transporter Cre02.g093700's role in paraquat tolerance and microcystin-related genes involved in cGMP/cAMP regulation. We employed multiple strategies to analyse our functional genomics dataset, including GSEA, manual exploration of top-responsive genes, and overrepresentation across all chemicals and specific chemical groups. While we have identified several genes with known and unknown functions that have an important role in tolerance and susceptibility to chemical exposure, the limited annotation of plant genes, particularly regarding their functional roles, makes it challenging to pinpoint the precise mechanisms of toxicity.

1.04.A.T-02 Tipping Points in the Lipidomes of Arctic Zooplankton Under Global Change

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Human activities induce extremely rapid environmental changes. This could exceed physiological tipping

points beyond which environmental changes have drastic impacts on organism's physiology. Tipping points have never been studied in the lipidome of organisms exposed to environmental changes. Yet, the lipidome plays a crucial role in the functioning of organism's physiology and ecosystems. Amongst the most striking example are Arctic copepods such as *Calanus glacialis*. Indeed, they store enormous lipid reserves, up to 60% of their body mass, and migrate to the deep sea to survive the winter in a state of metabolic dormancy, the diapause. Due to their energy-rich lipid composition, they play a key role in trophic food webs. Moreover, the winter migration of such lipid-rich organisms sequesters huge quantity of carbons in the deep ocean. The success of diapause is tightly related to changes in the lipidome. The Arctic ocean experiences Ocean Acidification (OA) four times faster than the global ocean. Changes in the lipidome and metabolic dormancy have been suggested to protect organisms when OA exceed tipping points. Thus, the lipidome lies at the interaction between diapause and the response to OA. Any changes in the lipidome of copepods would have consequences on the overwintering of copepods as well as on the functioning of Arctic ecosystems. Here we determine lipidome tipping points of organisms exposed to environmental changes. For that purpose, we exposed *C. glacialis*, a diapausing Arctic species, to 15 pH conditions for several weeks. In parallel, we exposed *Metridia longa*, a non-diapausing species that stays active over the winter. We expect the lipidome of *M. longa* to be affected only by OA while *C. glacialis* lipidome would reflect the interacting influences of diapause and OA. The lipid of the two species were analysed using targeted tandem mass spectrometry (MS/MS) liquid chromatography. We annotated ~500 lipid compounds using a combination of LOBSTAHS and MS-DIAL lipidomic pipelines. We then search for tipping points in the lipidome of both species using Weighted Gene Co-expression Network Analysis (WGCNA) and piecewise linear regressions. We also compare the lipidome reaction norms of both species using delta ranks to detect evolutionary mechanisms related to diapause. The method we develop will be applicable to a wide range of stressors and species and will be of broad interests for ecotoxicologists interested in lipids and contaminants.

1.04.A.T-03 Development of an In Vivo High Throughput Screening Approach for Mechanism-Based Toxicity Assessment of Plastic Additives Chemicals Using *Caenorhabditis elegans* Transcription Factor RNAi Library

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Identifying the toxic effects of brominated flame retardants (BFRs) contained in consumer products is crucial for accurately informing decision-makers in regulatory policies. RNA interference (RNAi) technology in *Caenorhabditis elegans* has been developed as a rapid and powerful screening method for identifying the toxicity pathways of various chemicals. Therefore, this study aims to facilitate the mechanistic understanding and identification of the toxic effects of BFRs using *C. elegans* RNAi high-throughput screening. In the first step, RNAi screening was performed for 384 *C. elegans* transcription factors (TFs). RNAi-fed worms were exposed to TBBPA, and their locomotion behavior was measured. The biological pathways triggered by the TFs that significantly led to either rescued or exacerbated toxicity were analyzed using KEGG and Reactome. Next, the toxicity pathways were validated using gene expression or mutants of *C. elegans* exposed to BFRs. Finally, a potential AOP with BFRs as stressors was constructed by utilizing the toxicological mechanisms associated with BFR exposure and the AOP Wiki. Among the 384 transcription factors that were screened for potential effects of TBBPA on *C. elegans* locomotion, 19% showed significant alterations in locomotion (45 genes; p -value < 0.05 , $2 < p$ -fold < 0.5). The associated pathways of these 45 transcription factors affected by TBBPA were revealed to be mostly involved in the nuclear receptor (NR) transcription pathway and retinoic acid signaling. The expression of NR transcription pathway-related genes, such as *nhr-69*, *nhr-8*, and *unc-55*, showed a statistically significant increase in *C. elegans* exposed to different concentrations of BFRs. To apply BFRs toxicity to an AOP framework, we aligned information on BFRs toxicity mechanisms, NR transcription pathway and retinoic acid signaling, with existing key events and adverse outcomes based on the AOP Wiki. In this study, we identified potential mechanisms of toxicity of BFRs using *C. elegans* RNAi library screening. This method has great potential as a fast and efficient screening method for mechanism-based toxicity assessment of chemicals, including flame retardants. This work was supported by Korea Environmental Industry & Technology Institute (KEITI) through 'Core Technology Development Project for Environmental Diseases Prevention and Management', funded by Korea Ministry of Environment (MOE) (2021003310005).

1.04.A.T-04 Impact of Simvastatin on *Gammarus locusta*: Metabolomic Measurement of Direct and Transgenerational Effects

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Simvastatin (SIM), a pharmaceutical widely prescribed for the treatment of hypercholesterolemia, is considered a contaminant of emerging concern based on its occurrence in aquatic systems and potential adverse effects in the environment and human health. Previous studies have already linked SIM inter/transgenerational exposure to disruption of neuroendocrine regulation pathways, concomitantly with reduced reproduction and growth in *Gammarus locusta*. To better understand the mechanistic into SIM's adverse effects and modes of action, metabolomics is presented as a powerful tool, offering valuable insights into organism's early molecular responses to stressors. In this study, untargeted ¹H NMR metabolomics was applied to assess the impact of SIM (at an environmentally relevant concentration) on the metabolism of *Gammarus locusta* males and females, considering both direct and transgenerational exposure. Results revealed the important gender-dependent nature of each of these effects. Directly exposed males showed enhanced glucose catabolism and tricarboxylic acid (TCA) cycle activity, in tandem with adaptations in osmotic regulation and glyoxylate metabolism. Exposed females exhibited only a small osmoregulatory effect. It is suggested that the response of exposed males may reflect previously reported high levels of methyl farnesoate hormone and alterations in apical factors, namely decreased growth. Conversely, transgenerational effects were identified only in females, with impact on energy metabolism (glycolysis and TCA cycle enhancement) and osmoregulatory response. This expresses the ability of female gametes to transmit the effects of direct SIM exposure. Such effects were putatively related to reported delayed maturation and transcriptomic deviations impacting on carbohydrate and lipid metabolisms, possibly specifically engaging phenylalanine/tyrosine in the catecholamine pathway. These findings highlight the importance of gender on the ability of responding and transmitting the effects of SIM through generations, offering critical information to improve hazard and risk assessment of biologically active compounds. The authors acknowledge TRANSEPIC ? Exploring Transgenerational Epigenetic Inheritance [Reference: 2022.02922.PTDC, doi: 10.54499/2022.02922.PTDC]; BetterBone [Reference: 2022.04286.PTDC, doi: 10.54499/2022.04286.PTDC], both financed by the FCT; project CICECO-Aveiro Institute of Materials UIDB/50011/2020 (doi: 10.54499/UIDB/50011/2020), UIDP/50011/2020 (doi: 10.54499/UIDP/50011/2020) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020), financed through the FCT/MCTES (PIDDAC); NMR spectrometer is part of the National NMR Network (PTNMR) supported by Infrastructure Project N° 022161.

1.04.P-We006 Cellular Handling, Transformation Pathways and Metabolic Disturbances of Mercury in Freshwater Phytoplankton

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Mercury, a priority pollutant, raises significant environmental concerns. While numerous studies have investigated mercury bioaccumulation and its impact on aquatic organisms, there is a gap in the understanding concerning its cellular handling and transformations, and disturbances of the metabolic pathways of phytoplankton species at the bottom of the food web. In such a context, this study aims to elucidate the mechanisms governing the cellular transformations and responses of phytoplankton to mercury exposure. The green alga *Chlamydomonas reinhardtii* and diatom *Cyclotella meneghiniana* were selected as representatives of major phytoplankton groups. These algae were exposed to 10 and 100 nM inorganic mercury (Hg(II)) and methylmercury (MeHg) for 72h. Mercury uptake, sub-cellular distribution, and physiological effects such as oxidative stress and photosynthetic yield were assessed. Simultaneously, over 90 metabolites, spanning antioxidants, amines, organic acids/phenolics, nucleobases/sides/tides, amino acids, sugars/sugar alcohols, and fatty acids, were quantified using liquid chromatography mass spectrometry. Cellular transformation including methylation/demethylation, reduction and oxidation as well as formation of HgS nanoparticles have been explored using double isotope Hg spiking (¹⁹⁹Hg(II) and ²⁰¹MeHg). Results demonstrated that *C. meneghiniana* exhibited higher mercury accumulation than *C. reinhardtii* at the same exposure concentrations. Subcellular distribution of mercury differed between the two species, with *C. reinhardtii* concentrating mercury predominantly in organelles, while the diatom exhibited mercury in both organelles and heat-stable peptides. Both algal species significantly demethylate methylmercury, whereas no measurable methylation of the inorganic mercury was found. A reduction of Hg(II) to Hg(0) were also found in both phytoplankton species. Physiological data matched metabolomic results, revealing substantial alterations in various pathways, including amino acids, nucleotides, fatty acids, the tricarboxylic acid cycle (TCA), and antioxidant metabolism. The findings reveal species-specific and concentration-dependent responses, providing new insights into mercury handling and

metabolic reprogramming in phytoplankton. Understanding these processes is essential for assessing mercury's impact on aquatic primary producers and its potential trophic transfer, especially in contaminated environments. The authors thank Swiss National Science Foundation grants 175721 and 180186 for the financial support.

1.04.P-We007 Towards Mechanistic Understanding of Reduced Cell Proliferation: An RNA- and RIBOseq Approach

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Fish are a highly diverse and ecologically important group of organisms that are exposed to a wide range of anthropogenic substances. While effects of toxic chemicals include both acute and chronic adverse outcomes, the latter seems to prevail in the natural environment. Therefore, ecotoxicological risk assessment is particularly interested in understanding the effects of prolonged chemical exposure to fish. One of the most often studied endpoints is organism growth. However, the assays usually conducted are not only under ethical scrutiny but also labour and time demanding. Therefore, new methods are desperately needed. While the OECD TG 249 already provides an alternative for the assessment of acute fish toxicity using the rainbow trout gill cell line, RTgill-W1, there is no such assay for chronic toxicity. However, building on the observation that larger animals have more cells instead of bigger ones, researchers from our group could demonstrate excellent predictive powers of the same cell line by taking cell number as proxy for organism size for a set of both structurally and functionally different chemicals. Nevertheless, the underlying molecular mechanisms and especially the points at which the different initially triggered biochemical pathways converge and lead to an arrest of the cell cycle remain unclear. To investigate the underlying molecular mechanisms, we exposed RTgill-W1 cells to a set of six different substances: 2-mercaptobenzothiazole, methimazole, propiconazole, tebuthiuron, topramezone and trifloxystrobin. While all six chemicals induced reduced cell proliferation, we found that derived EC50s correlate well with baseline toxicity QSARs. Next, to shed light on the underlying molecular mechanisms of reduced cell proliferation, we designed an experiment to analyse the transcriptome and translome. Gene ontology and pathway analysis using KEGG revealed a regulation of the cell cycle after an only one-day long exposure to the two chemicals already fully assessed. Specifically, gene ontology analysis of the RNAseq data showed that the cell cycle is affected during mitosis or at the G2/M checkpoint. Further, we identified several genes that were regulated differently at the level of transcription and translation. Altogether, the results will not only help us deciphering the mechanisms of reduced cell proliferation upon chemical insult but will also test the predictive powers of cell-line based assays.

1.04.P-We014 An Integrative Omics Approach for the Development of Conceptual Adverse Outcome Pathways: A Case Study Mapping Metabolic Syndrome in Tributyltin-Exposed Adipocytes

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Metabolic syndrome (MetS) is a condition distinguished by co-occurring symptoms that is increasingly attributed to exposure to endocrine-disrupting chemicals (EDCs). It is characterised by cardiometabolic irregularities and is associated with increased risk of cardiovascular disease (CVD), non-alcoholic fatty liver disease (NAFLD), and type 2 diabetes (T2D). Using an adverse outcome pathway (AOP) framework, this study leverages an integrated multi-omic approach using both univariate and multivariate techniques, as well as network and clustering analysis, to better visualise the intricate relationships between perturbed features (PFs), pathways, and disease associations. More specifically, Simpson-Golabi-Behmel syndrome (SGBS) pre-adipocytes were exposed to tributyltin (TBT), a known lipogenic substance, to assess metabolite and transcript dysregulation. Cells were grown to near confluence, incubated in differentiation medium for 4 days, and cultivated in maintenance medium for 6 days. The differentiation medium of TBT-exposed cells was additionally supplemented with 25nM TBT during the initial 4 days. On day 10, TBT-exposed cells and differentiated controls were compared. Samples for untargeted metabolomics were analyzed using Reversed Phase (RP) and Hydrophilic Interaction (HILIC) Liquid Chromatography in positive and negative ionization modes. Transcriptomic analysis was performed using Agilent microarrays to determine differentially expressed genes (DEGs). All data analyses took place in R. Data preprocessing, batch correction, and statistical analyses were conducted using xcms, IPO, PMCMRplus, and xMSannotator packages for metabolomics, and limma for transcriptomics. Pathway analysis was performed using KEGG, WikiPathway, and RaMP-DB databases in clusterProfiler and MetaboAnalystR while disease associations utilised DisGeNet. Cluster analysis using the Jaccard Similarity index was performed to group diseases and pathways based on their PF fingerprints. Six conceptual AOPs (cAOPs)

were developed that link disruptions in widespread pathway-level perturbances to adverse outcomes like xanthomatosis, bone remodeling disorders, cardiac outcomes, NAFLD, and insulin resistance. The resulting AOPs were also supported by recent literature findings. This method will be applied in future studies to explore environmental exposures and their contributions to various disorders, ultimately aiding in risk assessment and the development of targeted interventions.

1.04.B Molecular Ecotoxicology and Omics Perspectives: Advancing Mechanistic Understanding for Environmental Risk Assessment

1.04.B.T-01 Comparative Assessment of the tPOD of Tamoxifen in Zebrafish Embryos with Chronic Endpoints from a Two Generation Study

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The transcriptomic Point of Departure (tPOD) is gaining attention in ecotoxicology as a method for deriving quantitative endpoints from RNA-Seq data. In fish, the zebrafish embryo model is emerging as a promising New Approach Methodology (NAM), recognized under EU Directive 2010/63/EU as an alternative to traditional animal testing. Sublethal transcriptomic profiles in zebrafish embryos have been linked to various Modes of Action (MoA), with tPOD values from Fish Embryo Toxicity (FET) tests generally being protective and comparable, though often lower, than the No Observed Effect Concentration (NOEC) from chronic fish studies. However, there is a limited database to robustly suggest tPOD as a predictive tool for chronic endpoints derived from long-term fish tests. This study aimed to determine the tPOD for tamoxifen, an estrogen receptor modulator, in zebrafish embryos and compare it with endpoints from the Zebrafish Extended One Generation Reproduction Test (ZEOGRT). In the ZEOGRT, for the most sensitive endpoint for tamoxifen, the NOEC was determined to be 0.20 µg/L. Zebrafish embryos were exposed to tamoxifen concentrations (0.026 to 5.8 µg/L), covering the NOEC/LOEC obtained from the ZEOGRT. After 96 hours post-fertilization, RNA sequencing and differential expression analysis (DESeq2) were performed. The DRomics pipeline was used to identify the tPOD through transcriptomics benchmark dose (BMD) analyses in R. Significant concentration-dependent gene expression changes were observed, particularly in genes related to the endocrine system, such as Wnt signaling, MAPK signaling, and steroid hormone biosynthesis. Based on 64 responsive genes, tPOD values for tamoxifen were 10 ng/L (10th percentile) and 90 ng/L (maximum 1st peak), with the latter being slightly more sensitive than the ZEOGRT NOEC. These findings support the use of tPOD in the zebrafish FET test as a NAM for estimating the NOEC of tamoxifen, a pharmaceutical with endocrine activity. The slightly lower effect concentrations from this study compared to the NOEC from the ZEOGRT are in line with previous research and support that the tPOD is more conservative than apical points of departure. This study affirms that tPOD in the zebrafish embryo model could be a cost-effective, protective method for estimating NOECs, particularly for endocrine-disrupting chemicals, and supports the 3Rs principles in regulatory testing.

1.04.B.T-02 Sex Hormone Disruption of Biodegradable Plastic Extracts in Comparison with Conventional Plastics: Observations in H295R Cells and Adult Male Zebrafish

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Biodegradable plastics are generally considered a safer alternative to petroleum-based plastics. However, their endocrine-disrupting effects in comparison to those replaced remain unclear. We chose several plastic products with direct human contact, and assessed the endocrine-disrupting potential of biodegradable plastics using human adrenocortical carcinoma (H295R) cells and adult zebrafish. Eight products, including two with conventional plastics, were chosen, and their acetone extracts were used for exposure. In H295R cells, decreased testosterone (T), increased estradiol (E2), and upregulated cyp19a1, were observed following the exposure, suggesting anti-androgenic effects of most products. For in vivo observation, two samples with the highest in vitro responses were selected: a polybutylene adipate terephthalate (PBAT) + polylactic acid (PLA) sanitary bag and a high-density polyethylene (HDPE) sanitary bag as a control. Following a 21-day exposure to selected plastic extracts, adult male zebrafish exposed to PBAT+PLA extracts showed reduced 11-ketotestosterone (11-KT) levels, elevated E2 levels, and higher E2/11-KT ratios, supporting anti-androgenicity of these products. Gene expression analysis revealed upregulated cyp11a1, supporting enhanced estrogen synthesis, and upregulated fshr and lhr genes, indicating compensatory activation of the hypothalamic-pituitary-gonadal axis. The present observations in both in vitro and in vivo experiments clearly show that biodegradable plastics generally

exhibit the same directions of sex hormone disruption as conventional plastics. These findings underscore the need for stricter safety evaluations of biodegradable plastics of direct human contact to mitigate potential adverse health consequences. This work was supported by Korea Environmental Industry & Technology Institute (KEITI) through Core Technology Development Project for Environmental Diseases Prevention and Management, funded by Korea Ministry of Environment (MOE) (RS-2022-KE002173)

1.04.B.T-03 Disturbance in the Metabolic Pathways of Tree Frogs Living in the Chernobyl Exclusion Zone: From Gene, Protein Expression Patterns to Metabolic Enzyme Activity and Body Condition Index

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While wildlife is chronically exposed to various sources and levels of ionizing radiation in the environment, studies regarding the long-term impact of long-lived radio-contaminant released in case of accident, are still scarce, patchy, and sometimes contradictory. To increase such data, crucial to bring more realism to Ecological Risk Assessment, IRSN recent studies were performed on the tree frog *Hyla orientalis* living in the Chernobyl Exclusion Zone (CEZ) for ~15 generations. Analyses of genetic diversity and transcriptomics studies showed a high rate of mitochondrial mutations, a modulation of genes relative to energy metabolism, and, a small population size with a higher affiliation rate for those living in the most radio-contaminated areas. This raises questions on the ability of populations to maintain themselves and on their health status. In that way, to go further, proteomic analyses of frog muscles of the same individuals were performed and analyzed by two complementary methodologies, including dose-response curves. In addition, the activity of two enzymes involved in the energetic metabolism, i.e. citrate synthase (aerobic metabolism) and lactate dehydrogenase (anaerobic metabolism), was measured. All results were analyzed in relation to individual total dose rates (ITDR), distributed over five orders of magnitude. GO enrichment after proteomic analysis highlighted four significant enriched pathways (FDR 5%) i.e. Carboxylic acid metabolic process, Cellular lipid catabolic process, Fatty acid beta-oxidation and Small molecule catabolic process. Regarding enzyme activities, results showed a tendency of LDH activity decrease and a significant increase of the ratio between LDH and CS with increasing ITDR. All the results seem to confirm by two further approaches a disturbance in the metabolic pathways, notably the ones linked to fatty acids (?-oxidation, catabolism). Investigations will be carried on deciphering the biological pathways involved in long term effects of radiocontamination to draw hypotheses on the cascade of events leading to adverse outcome and conclude on CEZ tree frog health status.

1.04.B.T-04 Exploration of Transcriptomic Data with Over-Representation Analysis (ORA) Enhanced by Aggregated Biological Prior Knowledge

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In the context of transcriptomics, analyses yield extensive transcript lists that require impractical manual literature reviews. To address this, functional enrichment analysis, also known as pathway enrichment analysis, has become the standard approach. This method condenses large gene lists into more manageable and interpretable sets of biological functions or pathways (e.g. GO terms or KEGG pathways). One of the most common enrichment methods is the Over-Representation Analysis (ORA), which tests whether a biological function F contains a disproportionately large number of deregulated genes compared to sampling a random sample of expressed genes. In this case, the function F is considered as enriched. Enrichment analyses, while advantageous in highlighting certain functions, have the drawback of limiting interpretation to only a subset (often small) of the list of deregulated genes, specifically those involved in the enriched functions. We developed Cluefish (CLUstering, Enrichment and FISHing), a free, semi-automated workflow for exploring transcriptomic data. The workflow applies ORA to pre-clustered protein-protein interaction networks constructed from the STRING database, using clusters as anchors to identify smaller and more specific biological functions. Additionally, with steps for cluster merging and the retrieval of genes with shared biological context, Cluefish enables the inclusion of a larger portion of deregulated genes in the interpretation, providing a more comprehensive view of transcriptomic data. Using zebrafish embryos as an in vivo model, we investigated the dose-dependent transcriptional effects

of dibutyl phthalate (DBP). By comparing our approach with traditional methods, we demonstrated that Cluefish allows for the interpretation of a larger portion of the data and reveals disrupted biological pathways that would otherwise be overlooked. Coupled with sensitivity thresholds and dose-response curves derived from DROMICS, Cluefish outputs enabled the formulation of hypotheses supported by multiple concordant elements, which are not only biologically coherent but also of significant scientific interest.

1.04.P-We008 Metabolomic and Transcriptomic Changes in Larval Zebrafish After Developmental Exposure to Aroclor 1254

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Polychlorinated biphenyls (PCBs) are long-lived synthetic compounds that were widely used until 1979. The neurotoxicity of Aroclor 1254, a commercial mixture of PCBs, has been well documented within mammalian species, however, the underlying mechanisms of toxicity have not been fully characterized, particularly in aquatic organisms. Previous research has shown that developmental exposure to Aroclor 1254 induced dose dependent tremors in the eyes of larval zebrafish (*Danio rerio*), indicating novel neurotoxic effects. Here, we used targeted metabolomics and transcriptomic profiling to investigate the neurotoxicological effects of Aroclor 1254 exposure at nominal concentrations of 500 µg/L to embryonic zebrafish for 96 h. There were significant differences between controls and Aroclor 1254 treated zebrafish larvae in proline, glutarate, asparagine, glycerol 3-phosphate, sucrose, threonine, and fructose 6-phosphate levels. Metabolites involved in neurotransmitter signaling pathways, such as glycine, tyrosine, and glutamine, had significantly different levels in Aroclor 1254 treated larvae, with top enriched pathways involved in lipid metabolism and xenobiotic response. Bioinformatic analyses utilizing transcriptomic data found that exposure to Aroclor-1254 consistently resulted in disruptions of pathways and networks related to visual function, nervous system function, protein synthesis, and other pathways related to embryonic development. These underlying pathways were supported by eye tremor phenotypes that were present in Aroclor 1254 exposed larvae. These results suggest that changes in the levels of metabolites involved in neurotransmitter function and dysregulations in genes within visual function pathways are likely responsible for the underlying eye tremor behavior and may serve as novel biomarkers in early life stage fish exposed to PCBs.

1.04.P-We009 Ecotoxicogenomic Hazard Assessment of the Artificial Sweetener Sucralose

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The intensive consumption of artificial sweeteners has led to a global market revenue of more than 21 billion USD in 2021 and is expected to rise further. Mainly used as food additives, the high stability and water solubility of artificial sweeteners makes them perfect candidates as sugar alternatives. While the stability that comes with being calorie-free is an advantage in this case, it also makes them a potential threat to the environment due to the lacking degradation in waste water treatment plants. Consequently, they are released into the aquatic environment where they have proven to be persistent and mobile. Although researchers have described them as emerging contaminants for more than a decade, environmental hazard assessment data are still lacking. The few studies that have been carried out already revealed some ecotoxic effects such as neurotoxicity in zebrafish embryos caused by acesulfame or altered swimming behaviour of daphnids caused by sucralose. EFSA is currently re-evaluating the safety of artificial sweeteners, including environmental aspects. The use of OMICS for the required environmental hazard assessment offers many advantages, including time savings, insight into modes of action and reduction of animal testing. This project aims to gather data on the ecotoxicogenomic effects of the four most common artificial sweeteners sucralose, acesulfame, saccharin and cyclamic acid, using ecotoxicological model organisms from different trophic levels. First results showed that sucralose significantly altered gene expression of the aquatic plant *Lemna minor*, both, at the highest exposure of 100 mg/L and at the environmentally relevant concentration of 10 µg/L. Also, functional analysis provided hints on adverse effects caused by the substance. The Crustacean *Daphnia magna* and the teleost fish *Danio rerio* (embryo) were neither morphologically, nor molecularly affected by sucralose exposure in our experiments. The authors thank the German Federal Environmental Foundation for funding.

1.04.P-We030 Investigating the Effects of N-alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) on the Fathead Minnow (*Pimephales promelas*) Proteome, in a Two-Phase Approach and as Part of a Whole-Ecosystem Study

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N-alkyl dimethyl benzyl ammonium chloride (ADBAC) is a chemical disinfectant often integrated into household personal care products, surface cleaners, and cosmetics. Consumer ADBAC usage has significantly increased due to the ongoing COVID-19 pandemic. Individuals discharge these chemicals into wastewater, where the contaminants persist and become a threat to aquatic organisms. Although researchers have identified ADBACs in environmental water samples at $\mu\text{g/L}$ concentrations, few studies have examined their toxicological effects on fish. When fish are exposed to contaminants, they may develop molecular changes within their tissues and biofluids. Scientists can utilize proteomics to determine the effects of contaminants on protein abundance, and they can examine potential protein biomarkers that are tied to adverse outcome pathways. We aimed to utilize a proteomic approach to determine the effects of an ADBAC mixture on the fathead minnow (*Pimephales promelas*). We exposed 330 fathead minnows to 4 concentrations of a benzalkonium chloride mixture (0.02 $\mu\text{g/L}$, 0.2 $\mu\text{g/L}$, 2 $\mu\text{g/L}$, and 20 $\mu\text{g/L}$) and solvent control (0 $\mu\text{g/L}$) over a 28-day period. Following the ADBAC exposure, we anaesthetized the fish with 100 mg/L pH buffered tricaine methanesulfonate. Then, we collected mucus, plasma, and tissue samples. Next, proteins were sequenced with liquid chromatography-tandem mass spectrometry. Simultaneously, the International Institute of Sustainable Development Experimental Lakes Area (IISD-ELA) is conducting a multi-year, whole-lake ADBAC exposure. In this presentation, we will share the methodology from both phases of the study. Additionally, we will highlight the preliminary plasma and gill proteomics results from the laboratory exposure.

1.04.P Molecular Ecotoxicology and Omics Perspectives: Advancing Mechanistic Understanding for Environmental Risk Assessment

1.04.P-We001 Role of Nutritional Status on Arsenic Toxicity in *Daphnia pulex*: A Combined Life History and Multi-Omics Perspective on Individual and Interactive Effects

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Anthropogenic activities are readily introducing pollutants and altering nutrient availability into freshwater ecosystems. Activities such as mining, smelting, and fossil fuel extraction are releasing environmental pollutants such as arsenic, while degradation leads to alterations in crucial nutrients, such as phosphorus. Phosphorus is a critical nutrient for all organisms and alterations in their levels can lead to eutrophication. Food quantity (hereafter food) is crucial to organism metabolism and can influence toxicity of contaminants. While organisms within these environments experience these stressors simultaneously and influence toxicity of contaminants, there is limited research describing the interactive effect of multiple stressors on aquatic animals. Here we present a combined life history and multi-omics approach to evaluate the individual and interactive effects of food, food quality measured as phosphorus, and arsenic in aquatic keystone species, *Daphnia pulex*. We used a two-batch design for the multi-omic approach and to assess life history parameters. Batch A exposed *D. pulex* to arsenic (0.132 mg L⁻¹), while Batch B served as the control. Each batch was divided into treatments with high (3 mg C L⁻¹) or low (0.1 mg Carbon L⁻¹) food levels, and high (50 $\mu\text{mol P L}^{-1}$) or low (5 $\mu\text{mol P L}^{-1}$) phosphorus levels. Chronic exposure (40 days) to 0.132 mgC L⁻¹ arsenic under static renewal conditions was used. Differential gene expression analyzed using edgeR (p-value and FDR ≥ 0.05 , log₂ fold change ≥ 2). Gene Ontology (GO) and KEGG pathway analyses were conducted using topGO and our custom Pathway Enrichment Analysis Tool (PEAT). Metabolites were extracted from homogenized *Daphnia* and analyzed by mass spectrometry for protein identification. Results paralleled the life history and metabolomic findings with phosphorus alone and in conjugation with other factors resulted in the most significant effect. All data agree there was little arsenic effect. The interaction between phosphorus and arsenic exhibited the greatest differential gene expression with genes and pathways enriched for processes that affect development and energy production. There was no significance in the food and arsenic interaction. Our findings support that food, and phosphorus play differential roles in arsenic toxicity. This research demonstrates that coupling life history data with omics data, elucidates the complex connections between individual and sub-individual responses.

1.04.P-We002 Transcriptomic Fingerprinting of Pyriproxyfen and Abamectin Exposure in Zebrafish Embryos and Daphnia: Implications for Species Sensitivity and Risk Assessment

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The use of pesticides and biocides in agricultural and industrial applications raises significant concerns regarding their potential detrimental effects on aquatic ecosystems. These active substances can disrupt ecological balance, leading to adverse outcomes for both environmental health and biodiversity. Consequently, comprehensive environmental hazard and risk assessments are essential prior to the registration of any chemical substance. However, traditional risk assessment methodologies frequently overlook the importance of species-specific sensitivities. This oversight can lead to undetected long-term impacts, including developmental and behavioural disruptions. To address these challenges, this study employs transcriptomic analysis to investigate the molecular responses of two pesticides pyriproxyfen, a growth regulator acting as a juvenile hormone analog, and abamectin, a neurotoxic agent in zebrafish embryos and Daphnia, two widely utilized model organisms. Both species were exposed to sublethal concentrations of these pesticides. Although survival and hatching rates remained unaffected, transcriptomic analysis revealed distinct molecular responses. Exposure to pyriproxyfen significantly influenced genes related to hormonal regulation, neuronal development, and cellular processes associated with growth, suggesting potential endocrine disruption and developmental abnormalities. In contrast, abamectin primarily affected genes associated with mitochondrial function, oxidative stress, and neural apoptosis, indicating neurotoxic and metabolic disturbances. Notably, Daphnia exhibited greater sensitivity upon pyriproxyfen exposure compared to zebrafish embryos, underscoring species-specific differences in responses. Highlighting the elevated sensitivity of Daphnia relative to zebrafish embryos, this study advocates for a more refined understanding of pesticide impacts that considers species-specific responses. Such insights are crucial for developing robust risk assessment frameworks that accurately reflect the ecological risks associated with pesticide exposure, ultimately fostering responsible environmental stewardship.

1.04.P-We003 The Potential of OMICs in Anticipating and Differentiating Modes of Action of Fungicides

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Fungicides are widely applied to control fungal growth in agricultural and medicinal practices. However, due to their wide application, previous studies recorded residual concentrations of fungicides in the environment, making the contact between fungicides and environmental fauna and flora inevitable. Moreover, previous studies highlighted the adverse effects of fungicides with different modes of action (MoA) on non-target organisms. Together, the wide applications of fungicides along with their reported adverse effects on non-target organisms, highlight the importance of developing robust, cost and time-effective fungicidal hazard assessment approaches. In this study, we applied OMICs analysis to identify MoA-specific biomarker candidates, which may act as early indicators for ecotoxic MoA. First, the potential of OMICs approach to differentiate between two different MoAs was revealed by defining substance-specific biomarker candidates for two fungicides with two different MoAs, the sterol biosynthesis inhibitor difenoconazole (apoa1b, gatm, mylpfb and acta1b) and the nucleic acid metabolism inhibitor, metalaxyl (lgals2b, abat, fabp1b.1 and myh9a). Furthermore, we applied the approach to three azole fungicides with the same MoA (sterol biosynthesis inhibition). In addition to sterol biosynthesis inhibition in fungi, previous studies reported the endocrine disrupting effect of azole fungicides in fish. From the detected differentially expressed genes (DEGs), ugdh, gstt1b, pfkfb4b, fkbp5 and pnp4b were selected as biomarker candidates to predict endocrine disruption induced by sterol biosynthesis inhibition fungicide MoA in zebrafish embryos were defined. Additionally, we conducted functional analysis for the detected DEGs and integrated the obtained information to understand the potential ecotoxic MoA of fungicides in non-target organisms and study the relation between the detected molecular responses and the original MoA of the tested fungicide in the target organism according to fungicide resistance action committee (FRAC) classification. The defined biomarker candidates will act as pre-regulatory screening approach to anticipate ecotoxic MoAs of different classes of chemical substances in compliance to the 3Rs principle by providing early indications of the ecotoxic MoAs not approved for application in the European union such as endocrine disruption.

1.04.P-We004 A Transcriptomic Points of Departure Assay for Rainbow Trout Embryos: Comparisons with Fish Acute and Chronic Toxicity Data

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There is regulatory, scientific, and ethical interest in the development of alternatives to vertebrate animal toxicity tests. The objective of this study was to establish a high-throughput test method for embryo-larval fish that could yield data comparable to both fish acute (i.e., 96-hr LC50s) and chronic (i.e., long-term, sub-lethal) toxicity tests. Rainbow trout alevins (~1 day post-hatch) were placed in individual wells (24-well plates) and exposed for 24 hr to 12 different concentrations per chemical. To date, 39 chemicals with diverse physical and chemical properties have been studied, including negative controls (fish medium water or 0.5% DMSO). To eliminate the need for a range-finding test, test concentrations were based on a tapered design which included an LC50 push zone (10- and 100-fold higher than the empirical LC50s listed in the U.S. EPA ECOTOX Knowledgebase), transcriptomic point of departure (tPOD) zone (six concentrations below the LC50 range on a half log10 basis), and baseline zone (10- and 100-fold lower concentrations). Using this design, the exposure for ethanol was: 1000, 100, 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.001, 0.0001, and 0 mg/L (covering 7 orders of magnitudes). Mortality during transport, rearing, hatching, and experimental phases were all lower than guidance from OECD236. To ensure repeatability of the results, copper sulfate was studied four times and yielded consistent LC50 values. Based on current data, the relationship between LC50 data from our work and that of fish acute studies (from databases) is near 1:1 with a r^2 of about 0.9. A tPOD has been derived for six compounds (with sequencing data in hand for 18 more), and in all cases the tPOD concentration seems to be protective (i.e., lower than, or similar to) of values associated with sub-lethal outcomes from chronic bioassays. The findings thus far are promising in terms of establishing a new test method, though work continues to expand our sequencing efforts to yield more tPOD data, test more compounds, and deepen studies into exposure measures (i.e., nominal vs. modeled vs. measured). The work was funded by Genome Canada/Genome Quebec s GAPP program.

1.04.P-We005 Contribution of Dose-Response Modelling for Mechanistic Understanding of (Multi-)Omics Data and Risk Assessment

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Omics technologies has opened new possibilities to assess environmental risks and to understand the mode(s) of action of pollutants from the molecular level. Given their cost, they are usually employed in experimental designs where few conditions (e.g. 2 to 4 exposure concentrations) are compared to a control condition through differential analysis. However, in such studies, the results observed in each condition (up or down-regulations compared to the control, and fold change) are derived independently, hence limiting the relevance for risk assessment. In contrast, when coupled to dose-response experimental designs, omics technologies allow a non-targeted assessment of organism responses along an exposure gradient. Though describing the dose-response relationships on high-throughput data is no easy task, tools are now available for exploiting dose-response omics data (e.g. DRomics, FastBMD, BMDExpress), making use of the information all along the exposure gradient at each step. To begin, (1) they identify the items (e.g. transcripts, metabolites) that are significantly deregulated along the dose/concentration gradient. Then for each deregulated item, they (2) characterize its dose-response relationship using parametric modelling and (3) estimate from this model a sensitivity threshold as a benchmark dose (BMD). Resulting outputs synthesize the responses all along the exposure gradient and are full of meaning for risk assessment. Besides, in the light of biological annotation obtained from databases such as KEGG or Gene Ontology (GO), it is possible to look at pathway-wise points of departure towards a mechanistic understanding of dose-dependent omics data. After presenting the landscape of omics data analysis in (eco)toxicology, we will give an overview of the methodological choices we made in developing the DRomics tool. Our purpose was to rationalize and optimize a strategy to fully exploit dose-response omics data, not only focusing on monotonic responses (increasing, decreasing) but also biphasic responses (U and bell-shape) that are often encountered on omics. We will also illustrate on different case studies some functionalities recently added in DRomics to help the comparison of responses obtained at different experimental levels: different measurement levels (e.g. multi-omics), different exposure histories, time points, etc. Finally, we will discuss pending methodological challenges and open questions.

1.04.P-We006 Cellular Handling, Transformation Pathways and Metabolic Disturbances of Mercury in Freshwater Phytoplankton

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Mercury, a priority pollutant, raises significant environmental concerns. While numerous studies have investigated mercury bioaccumulation and its impact on aquatic organisms, there is a gap in the understanding concerning its cellular handling and transformations, and disturbances of the metabolic pathways of phytoplankton species at the bottom of the food web. In such a context, this study aims to elucidate the mechanisms governing the cellular transformations and responses of phytoplankton to mercury exposure. The green alga *Chlamydomonas reinhardtii* and diatom *Cyclotella meneghiniana* were selected as representatives of major phytoplankton groups. These algae were exposed to 10 and 100 nM inorganic mercury (Hg(II)) and methylmercury (MeHg) for 72h. Mercury uptake, sub-cellular distribution, and physiological effects such as oxidative stress and photosynthetic yield were assessed. Simultaneously, over 90 metabolites, spanning antioxidants, amines, organic acids/phenolics, nucleobases/sides/tides, amino acids, sugars/sugar alcohols, and fatty acids, were quantified using liquid chromatography mass spectrometry. Cellular transformation including methylation/demethylation, reduction and oxidation as well as formation of HgS nanoparticles have been explored using double isotope Hg spiking ($^{199}\text{Hg(II)}$ and $^{201}\text{MeHg}$). Results demonstrated that *C. meneghiniana* exhibited higher mercury accumulation than *C. reinhardtii* at the same exposure concentrations. Subcellular distribution of mercury differed between the two species, with *C. reinhardtii* concentrating mercury predominantly in organelles, while the diatom exhibited mercury in both organelles and heat-stable peptides. Both algal species significantly demethylate methylmercury, whereas no measurable methylation of the inorganic mercury was found. A reduction of Hg(II) to Hg(0) were also found in both phytoplankton species. Physiological data matched metabolomic results, revealing substantial alterations in various pathways, including amino acids, nucleotides, fatty acids, the tricarboxylic acid cycle (TCA), and antioxidant metabolism. The findings reveal species-specific and concentration-dependent responses, providing new insights into mercury handling and metabolic reprogramming in phytoplankton. Understanding these processes is essential for assessing mercury's impact on aquatic primary producers and its potential trophic transfer, especially in contaminated environments. The authors thank Swiss National Science Foundation grants 175721 and 180186 for the financial support.

1.04.P-We007 Towards Mechanistic Understanding of Reduced Cell Proliferation: An RNA- and RIBOseq Approach

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Fish are a highly diverse and ecologically important group of organisms that are exposed to a wide range of anthropogenic substances. While effects of toxic chemicals include both acute and chronic adverse outcomes, the latter seems to prevail in the natural environment. Therefore, ecotoxicological risk assessment is particularly interested in understanding the effects of prolonged chemical exposure to fish. One of the most often studied endpoints is organism growth. However, the assays usually conducted are not only under ethical scrutiny but also labour and time demanding. Therefore, new methods are desperately needed. While the OECD TG 249 already provides an alternative for the assessment of acute fish toxicity using the rainbow trout gill cell line, RTgill-W1, there is no such assay for chronic toxicity. However, building on the observation that larger animals have more cells instead of bigger ones, researchers from our group could demonstrate excellent predictive powers of the same cell line by taking cell number as proxy for organism size for a set of both structurally and functionally different chemicals. Nevertheless, the underlying molecular mechanisms and especially the points at which the different initially triggered biochemical pathways converge and lead to an arrest of the cell cycle remain unclear. In order to investigate the underlying molecular mechanisms, we exposed RTgill-W1 cells to a set of six different substances: 2-mercaptobenzothiazole, methimazole, propiconazole, tebuthiuron, topramezone and trifloxystrobin. While all six chemicals induced reduced cell proliferation, we found that derived EC50s correlate well with baseline toxicity QSARs. Next, in order to shed light on the underlying molecular mechanisms of reduced cell proliferation, we designed an experiment to analyse the transcriptome and translome. Gene ontology and pathway analysis using KEGG revealed a regulation of the cell cycle after an only one-day long exposure to the two chemicals already fully assessed. Specifically, gene ontology analysis of the RNAseq data showed that the cell cycle is affected during mitosis or at the G2/M checkpoint. Further, we identified several genes that were regulated differently at the level of transcription and translation. Altogether, the results will not only help us deciphering the mechanisms of reduced cell proliferation upon chemical insult but will also test the predictive powers of cell-line based assays.

1.04.P-We008 Metabolomic and Transcriptomic Changes in Larval Zebrafish After Developmental Exposure to Aroclor 1254

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Polychlorinated biphenyls (PCBs) are long-lived synthetic compounds that were widely used until 1979. The neurotoxicity of Aroclor 1254, a commercial mixture of PCBs, has been well documented within mammalian species, however, the underlying mechanisms of toxicity have not been fully characterized, particularly in aquatic organisms. Previous research has shown that developmental exposure to Aroclor 1254 induced dose dependent tremors in the eyes of larval zebrafish (*Danio rerio*), indicating novel neurotoxic effects. Here, we used targeted metabolomics and transcriptomic profiling to investigate the neurotoxicological effects of Aroclor 1254 exposure at nominal concentrations of 500 µg/L to embryonic zebrafish for 96 h. There were significant differences between controls and Aroclor 1254 treated zebrafish larvae in proline, glutarate, asparagine, glycerol 3-phosphate, sucrose, threonine, and fructose 6-phosphate levels. Metabolites involved in neurotransmitter signaling pathways, such as glycine, tyrosine, and glutamine, had significantly different levels in Aroclor 1254 treated larvae, with top enriched pathways involved in lipid metabolism and xenobiotic response. Bioinformatic analyses utilizing transcriptomic data found that exposure to Aroclor-1254 consistently resulted in disruptions of pathways and networks related to visual function, nervous system function, protein synthesis, and other pathways related to embryonic development. These underlying pathways were supported by eye tremor phenotypes that were present in Aroclor 1254 exposed larvae. These results suggest that changes in the levels of metabolites involved in neurotransmitter function and dysregulations in genes within visual function pathways are likely responsible for the underlying eye tremor behavior and may serve as novel biomarkers in early life stage fish exposed to PCBs.

1.04.P-We009 Ecotoxicogenomic Hazard Assessment of the Artificial Sweetener Sucralose

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The intensive consumption of artificial sweeteners has led to a global market revenue of more than 21 billion USD in 2021 and is expected to rise further. Mainly used as food additives, the high stability and water solubility of artificial sweeteners makes them perfect candidates as sugar alternatives. While the stability that comes with being calorie-free is an advantage in this case, it also makes them a potential threat to the environment due to the lacking degradation in waste water treatment plants. Consequently, they are released into the aquatic environment where they have proven to be persistent and mobile. Although researchers have described them as emerging contaminants for more than a decade, environmental hazard assessment data are still lacking. The few studies that have been carried out already revealed some ecotoxic effects such as neurotoxicity in zebrafish embryos caused by acesulfame or altered swimming behaviour of daphnids caused by sucralose. EFSA is currently re-evaluating the safety of artificial sweeteners, including environmental aspects. The use of OMICs for the required environmental hazard assessment offers many advantages, including time savings, insight into modes of action and reduction of animal testing. This project aims to gather data on the ecotoxicogenomic effects of the four most common artificial sweeteners sucralose, acesulfame, saccharin and cyclamic acid, using ecotoxicological model organisms from different trophic levels. First results showed that sucralose significantly altered gene expression of the aquatic plant *Lemna minor*, both, at the highest exposure of 100 mg/L and at the environmentally relevant concentration of 10 µg/L. Also, functional analysis provided hints on adverse effects caused by the substance. The Crustacean *Daphnia magna* and the teleost fish *Danio rerio* (embryo) were neither morphologically, nor molecularly affected by sucralose exposure in our experiments. The authors thank the German Federal Environmental Foundation for funding.

1.04.P-We010 Sex-Specific Behavioural Ecotoxicology and Transcriptomic Responses to Phenanthrene in the Amphipod *Parhyale hawaiiensis*

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Polycyclic aromatic hydrocarbons (PAHs) are pervasive environmental contaminants that disrupt critical biological processes in marine organisms and pose significant risks to ecosystem health. Phenanthrene (PHE), a model PAH, exhibits gender-specific behavioural toxicity, yet the molecular mechanisms underlying these effects remain poorly understood. The objective of this study was to investigate

molecular mechanisms of behavioural ecotoxicity in the model tropical marine amphipod *Parhyale hawaiiensis*. First, we determined the 96-hour LC50 of PHE and assessed the seven-day sublethal gender-specific behavioural impacts of PHE on adult *P. hawaiiensis*. Organisms were subsequently exposed to a sublethal PHE concentration (100 µg/L) alongside solvent (DMSO) and seawater controls for transcriptomic profiling. Whole amphipods of each gender were collected at 0, 1, 4, 8, and 24 hours for RNA sequencing (RNA-seq) and to capture time- and sex-specific gene expression changes. Our findings reveal significant alterations in detoxification enzymes, neurotransmitter receptors, and heat shock proteins, with temporal variations in expression patterns. These results demonstrate the sensitivity of transcriptomics for revealing molecular responses to PHE exposure and their connection to behavioural disruptions. This integrative approach highlights the importance of combining molecular and behavioural analyses to improve ecological risk assessment and develop sensitive biomarkers for PAH toxicity. Lawan, I., was supported by the Petroleum Technology Development Fund (PTDF) Nigeria for academic sponsorship. The funder had no role in study design, data collection and analysis, decision to publish, or abstract preparation.

1.04.P-We011 Metabolomics Analysis for the Investigation of New Endocrine-Sensitive Endpoints in the Great Pond Snail *Lymnaea stagnalis*

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Aligned with the 3Rs principles, the use of invertebrates as model organisms is one of the possible new approach methodologies (NAMs) investigated in ecotoxicological testing. The great pond snail *Lymnaea stagnalis* is a promising model organism for the risk assessment of endocrine-disrupting chemicals (EDCs). However, important gaps remain in the understanding of the endocrine system of mollusks and other invertebrate species. Elucidating the potential endocrine-disrupting mechanisms of compounds of interest requires a foundational understanding of the cascade of biological events, from the molecular to the organism level. The elaboration of adverse outcome pathways (AOPs) is key in this process, and omics approaches can help to link observed adverse effects, to molecular mechanisms. In this study, we want to investigate the presence and the role of vertebrate-like thyroid hormones (THs) and steroid hormones (SHs) in the great pond snail *L. stagnalis*. Egg and tissue samples from our snail colony in Denmark (wild type, Danish origin) were collected and sent to the UVic Genome BC Proteomics Center in Victoria, BC, Canada, for analysis. After reception, samples < 100 mg were directly homogenized by bead beating in a DTT/ascorbic acid/citric acid (5:10:10 mg/mL) buffer, and samples > 100 mg were ground into powder using liquid nitrogen in a mortar and pestle prior to homogenization. Hormones were extracted by liquid-liquid extraction with ethyl acetate (EA). After centrifugation, the EA phase was dried under a flow of nitrogen and samples were reconstituted in MeOH/H₂O (40:60 v/v). The LC-MS/MS system consisted in a reversed-phase C18 column (2.1 x 50 mm, 1.8 µm particle size, Agilent Technologies), hyphenated with a triple-quadrupole system (Agilent 6495B). Our first results suggest that we are able to detect certain THs and SHs in egg or tissue samples. Although the role of such hormones in invertebrates has yet to be clearly understood, our findings are aligned with results already published about THs and SHs in invertebrate species. We also are, to our knowledge, the firsts to use a highly sensitive and selective detection method to detect simultaneously THs and SHs in wild-type *L. stagnalis*.

1.04.P-We012 Disruption of Thyroid and Sex Steroid Hormones by Trioctyl Trimellitate in Zebrafish: Integrating Mechanistic and Transcriptomic Analysis

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Tris(2-ethylhexyl) trimellitate (TOTM) is a widely used alternative plasticizer to replace bis(2-ethylhexyl) phthalate (DEHP), especially in medical environments. Although considered safer than DEHP, studies on TOTM's toxicity remain limited. This study investigated the effects of TOTM on thyroid hormones, lipid metabolism, and sex steroid hormones in both embryo-larval and adult male zebrafish. Fertilized zebrafish eggs were exposed to TOTM (0, <0.047, 0.42, and 5.00 mg/L) for seven days and were evaluated for changes in thyroid hormone levels, transcriptional activity of thyroid-regulating genes, and potential disruptions in lipid metabolism and related gene expressions. Transcriptomic analysis was also performed on 7-day post-fertilization (dpf) zebrafish larvae following exposure to 0 and 5.00 mg/L TOTM. Additionally, adult male zebrafish were exposed to TOTM (0, <0.047, and 0.38 mg/L) for 21 days, where alterations in thyroid and sex steroid hormone levels and corresponding transcriptional changes were assessed. In 7 dpf zebrafish larvae, exposure to TOTM significantly increased whole-body TT4 and TT3 levels, and down-regulated genes such as *crh*?, *trh*, *mct8*, and *nkx2.1b*. In the transcriptomics analysis, a

total of 1,380 differentially expressed genes (DEGs) were identified, with an adjusted p-value below 0.05; 402 DEGs were up-regulated, and 978 DEGs down-regulated. Gene ontology (GO) analysis showed that up-regulated DEGs were mainly associated with RNA splicing, while down-regulated DEGs were linked to disrupted cholesterol homeostasis and steroid metabolic process. In adult male zebrafish, exposure to TOTM resulted in a significant decreasing trend in TT3 levels accompanied by up-regulation of tg, tpo, nkx2.1b, and dio3a genes in the thyroid gland, as well as down-regulation of the dio2 gene in the liver. Additionally, a significant increase in E2 levels with a decrease in 11-KT levels was observed, accompanied by up-regulation of genes such as vtg1, vtg2, and cyp19a1.

While making a direct comparison with other studies may be challenging due to variations in exposure periods and conditions, the current observations suggest that this alternative plasticizer could alter hormonal balances at different life stages of zebrafish. Consequences in their normal development and reproduction warrant further investigations. This work was supported by Korea Environmental Industry & Technology Institute (KEITI) through Core Technology Development Project for Environmental Diseases Prevention and Management, funded by Korea Ministry of Environment (MOE) (RS-2022-KE002173)

1.04.P-We013 Association Between Exposure to Environmental Pollutants and Non-Alcoholic Fatty Liver Disease (NAFLD) and Development of Hepatotoxicity Biomarker Using Zebrafish

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Non-alcoholic fatty liver disease (NAFLD), characterized by over 5% fat accumulation in the liver without excessive alcohol use or other liver conditions, is increasingly prevalent among adolescents. Traditionally diagnosed through invasive liver biopsies, efforts are underway to improve non-invasive methods using biomarkers and imaging tools. This study explored the association between urinary metabolites of bisphenol A (BPA) and di-(2-ethylhexyl) phthalate (DEHP) with NAFLD in adolescents using data from the 3rd Korean National Environmental Health Survey (KoNEHS) and evaluated potential biomarkers in zebrafish models. Data from 831 adolescents aged 12-17 years were analyzed, calculating urinary BPA and DEHP metabolites (MEHHP, MEOHP) and classifying NAFLD based on serum alanine aminotransferase (ALT) levels (≥25 U/L for males, ≥22 U/L for females). Zebrafish embryos were exposed to BPA and DEHP (0.03-300 µg/L), and enzyme-linked immunosorbent assay (ELISA) and real-time PCR were used to assess liver enzymes and expression of ER stress and apoptosis genes. Results showed that NAFLD was more prevalent among overweight or obese males. Higher urinary BPA and MEHHP levels correlated with elevated ALT, suggesting an increased NAFLD risk from environmental exposure. In zebrafish, BPA and DEHP exposure reduced body length and increased ALT activity at 30-300 µg/L. Significant upregulation of chop, baxa, and bcl2a genes indicated ER stress and apoptosis, potentially linking these chemicals to liver damage. This study highlights the hepatotoxic effects of BPA and DEHP, identifying chop, baxa, and bcl2a as potential biomarkers for detecting NAFLD induced by environmental pollutants. These findings underscore the need to monitor and regulate BPA and DEHP exposure to protect adolescent health. This work was supported by National Research Foundation of Korea (NRF) grant funded by the Ministry of Science and ICT (MSIT) of South Korea (RS-2023-00251751).

1.04.P-We014 An Integrative Omics Approach for the Development of Conceptual Adverse Outcome Pathways: A Case Study Mapping Metabolic Syndrome in Tributyltin-Exposed Adipocytes

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Metabolic syndrome (MetS) is a condition distinguished by co-occurring symptoms that is increasingly attributed to exposure to endocrine-disrupting chemicals (EDCs). It is characterised by cardiometabolic irregularities and is associated with increased risk of cardiovascular disease (CVD), non-alcoholic fatty liver disease (NAFLD), and type 2 diabetes (T2D). Using an adverse outcome pathway (AOP) framework, this study leverages an integrated multi-omic approach using both univariate and multivariate techniques, as well as network and clustering analysis, to better visualise the intricate relationships between perturbed features (PFs), pathways, and disease associations. More specifically, Simpson-Golabi-Behmel syndrome (SGBS) pre-adipocytes were exposed to tributyltin (TBT), a known lipogenic substance, to assess metabolite and transcript dysregulation. Cells were grown to near confluence, incubated in differentiation medium for 4 days, and cultivated in maintenance medium for 6 days. The differentiation medium of TBT-exposed cells was additionally supplemented with 25nM TBT during the initial 4 days. On day 10, TBT-exposed cells and differentiated controls were compared. Samples for untargeted metabolomics were analyzed using Reversed Phase (RP) and Hydrophilic Interaction (HILIC) Liquid Chromatography in positive and negative ionization modes. Transcriptomic analysis was performed using Agilent microarrays

to determine differentially expressed genes (DEGs). All data analyses took place in R. Data preprocessing, batch correction, and statistical analyses were conducted using xcms, IPO, PMCMRplus, and xMSannotator packages for metabolomics, and limma for transcriptomics. Pathway analysis was performed using KEGG, WikiPathway, and RaMP-DB databases in clusterProfiler and MetaboAnalystR while disease associations utilised DisGeNet. Cluster analysis using the Jaccard Similarity index was performed to group diseases and pathways based on their PF fingerprints. Six conceptual AOPs (cAOPs) were developed that link disruptions in widespread pathway-level perturbances to adverse outcomes like xanthomatosis, bone remodeling disorders, cardiac outcomes, NAFLD, and insulin resistance. The resulting AOPs were also supported by recent literature findings. This method will be applied in future studies to explore environmental exposures and their contributions to various disorders, ultimately aiding in risk assessment and the development of targeted interventions.

1.04.P-We015 Toxicophenomic Assessment of the Combined Effect of Metsulfuron-methyl Exposure and Drought on Sinapis Arvensis

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The integration of the effect of climate change is a pressing challenge for risk assessment (RA), as traditional methods are inadequate for evaluating multiple stressors. Fortunately, new data types emerge and, with them, the potential to tackle this challenge and improve RA. For example, toxicophenomics, defined as the application of phenotyping techniques in ecotoxicological studies, can yield data on hundreds of endpoints over time, allowing to dynamically capture the stress responses. This study aims to investigate the combined effects of metsulfuron-methyl exposure and drought on wild mustard (*Sinapis Arvensis*) over time using toxicophenomic data. We hypothesise that toxicophenomics data will provide additional knowledge compared to traditional methods, making it a leverage point to develop the next generation of approaches in RA. The experiment was conducted on wild mustard (*Sinapis arvensis*). Plants were sprayed with increasing doses of the herbicide metsulfuron-methyl and were subjected to three different drought regimes. The plants were monitored in a fully automated high-throughput phenotyping platform for three weeks after spraying. The measurements consisted of daily non-destructive measurements and destructive measurements at the end of the experiment. The data will be analysed by fitting dose-response models and extracting parameters of interest, such as the benchmark dose. Multispectral imaging will yield reflectance data, which will be used to calculate vegetation indices, and fluorescence data, which, in combination with the molecular analyses of secondary metabolites, will allow us to identify bio-signatures and assess early response to drought stress. Other non-destructive measurements will include the number of yellow pixels per image, thermal imaging, and polyphenol content. Destructive measurements include dry weight, height, and leaf area. The benchmark doses will allow us to compare the impact of herbicide dose under different drought regimes across endpoints and over time, which is a major improvement compared to previous studies which compare one endpoint at one time point. This study will shed light on the potential of toxicophenomic data to effectively assess multiple stressors and will guide the development of RA towards more reliable predictions of environmental outcomes. The methods and analysis can be applied to other chemicals, stressors, endpoints and species, making it relevant to many fields within RA. This work was supported by the Novo Nordisk Foundation.

1.04.P-We016 Evaluating the Potential of Bioactive Metabolites in Red Seaweed Extracts: Addressing Key Challenges Through Integration and Innovation of Cell Assays

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Food security is one of the biggest challenges in the 21st century. As the focus of food requirements shifts towards enhancing human health and preventing disease. Seaweed has garnered significant attention as a natural source of bioactive compounds but also as a source of environmental contaminants when cultured in or harvested from the field. However, current research provides little insight into the effects of entire seaweed extracts which contain complex mixtures of bioactive ingredients with potential environmental pollutants, nor do we fully understand the molecular mechanisms through which they act in human cells. This knowledge gap limits our ability to assess the true potential of seaweed and consequences of increased environmental pollution. Therefore, we explored the use of novel approach methodologies to evaluate the impact of both purified compounds derived from red seaweed on human cell lines, while also examining whole seaweed extracts for a comprehensive analysis. The red pigment R-phycoerythrin (R-PE) and the carbohydrate floridoside were evaluated, while aquatic seaweed extracts (in PBS solution) from three red algal species, i.e. *Porphyra umbilicalis*, *Acrochaetium secundatum* and *Gracilaria gracilis* were explored. Cytotoxic, antioxidant, and metabolic properties, as early biomarkers of adversity, were evaluated towards two human cell lines: colon adenocarcinoma Caco-2 cells and the acute monocytic leukemia cell line THP-1. Cell viability, metabolic, cytokine and ROS measurements were performed on

THP-1 and Caco-2 after exposure to the different treatments. As such, the integration of these data will allow us to improve our understanding of the specific roles of the molecules of interest in the corresponding extracts, which will be crucial for AOP development. Through this research, we aim to advance both the aquaculture as food safety fields by establishing quantitative links between molecular markers and the chemical composition of seaweed.

1.04.P-We017 De Novo Transcriptome Assembly and Annotation of *Gammarus pulex*: A Valuable Resource For Ecotoxicogenomics

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This study presents high-quality de novo transcriptome assemblies for *Gammarus pulex*, a freshwater amphipod used in ecotoxicological research. These novel transcriptomes will allow the exploration of molecular responses (i.e., biomarkers, metabolic pathways, and molecular diversity) to chemical pollution. Transcriptomes were assembled from specimens collected in streams and small rivers featuring distinct loads of micropollutants, including pesticides, pharmaceuticals, and industrial chemicals in Sweden and Germany. We generated three assemblies: German (DE), Swedish (SWE), and a combined DE-SWE assembly. Quality validation removed a substantial number of low-confidence sequences, enhancing the fidelity of the final assemblies. Taxonomic analysis showed over 90% of assignments attributed to eukaryotic taxa. The DE-SWE assembly captured the highest gene and transcript diversity, supporting a broad representation of biological pathways. Annotation revealed extensive functional information, with the DE-SWE assembly containing 8.8% of sequences with unique annotations and displaying enriched metabolic diversity. Functional pathway enrichment analyses highlighted conserved biological processes, including organonitrogen compound metabolism and intracellular transport. We observed unique enriched pathways which were site-specific. Molecular function analyses revealed catalytic activity and protein binding as dominant roles across assemblies, with greater diversity in DE-SWE. This dataset provides a valuable resource for ecotoxicogenomic research, offering comprehensive gene models and pathways that enhance understanding of *G. pulex* responses to environmental stressors and inform aquatic pollution management. Funding by the Swedish Research Council FORMAS is gratefully acknowledged (project MixTOX, grant No. 2020-00976). Additional funding was provided by the FRAM Centre for Future Risk Assessment and Management Strategies at the University of Gothenburg.

1.04.P-We018 AQUADIVERS: Employing Metabarcoding to Assess the Local Impact of Metal Emissions on Freshwater Biodiversity

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Environmental monitoring of chemicals compares contaminant thresholds set by ecotoxicological testing with measured concentrations to avoid impacts on biodiversity. Ex-situ ecotoxicological assays typically use isolated pollutants on single-species individuals, which do not capture the complexity of natural communities, their interspecies interactions, or the combined effects of multiple pollutants. Moreover, traditional taxonomic approaches for biodiversity assessments come with limitations, including being impractical, not cost-effective, prone to misidentification, and insufficient consideration of whole-community impacts or key trophic and functional dynamics essential to ecosystem health. The AQUADIVERS project aims to address these gaps by assessing freshwater quality and biodiversity (focusing on eukaryotic organisms) at sites upstream and downstream of emission points of metal industry plants in Flanders, Belgium, while also examining additional impacts (e.g. on taxa richness) on freshwater ecosystems. This study employed a community-based approach, leveraging high-throughput environmental DNA (eDNA) metabarcoding techniques, which rely on the amplification and sequencing of genetic markers to identify multiple species within a sample. This approach moves beyond conventional monitoring methods and enable comprehensive biodiversity analysis. Alongside standard water quality measures, which included metal concentrations, organic carbon, and nutrients, this research investigated the eukaryotic microbial diversity within stone and glass periphyton (18S rRNA gene metabarcoding) at stations upstream and downstream of metal industry effluent discharge. The abundance of eukaryotic taxa, particularly diatoms (in line with regulatory guidelines) was used to calculate biodiversity indices for each site. Studying the relationship between taxa abundances, biodiversity indices and environmental stressors (e.g. metal concentrations) aided in identifying potential metal-sensitive and metal-resistant taxa and indicating if metal emissions were disrupting the local biodiversity. This highlights how metabarcoding, with its ability to detect diverse taxa and quantify their responses to

environmental pressures, can provide a powerful tool for biodiversity assessments, offering detailed insights into ecosystem health. This project is funded by Eurometaux and VLAIO (Flemish Agency for Innovation and Entrepreneurship).

1.04.P-We019 Aerial eDNA – A New Approach to Identify and Monitor Species in Farmland: A Pilot Study

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All species leave DNA traces in the environment. Environmental DNA (eDNA) is released by any organism into the environment, via breathing, faeces, hair, urine, skin, gametes, etc. This eDNA can be extracted from environmental samples such as soil, water or faeces without capturing potential target organisms in focus, even if it is not known whether the target organism is occurring in the sample area. More recently, studies have revealed that air also serves as a medium for transporting DNA. Approaches focussing on eDNA to monitor species are now widely recognized as a valuable tool for Biodiversity assessments. In our study conducted in a farmland landscape in southwestern Germany. We investigated the potential of aerial eDNA sampling for identifying species or specific taxonomic groups within the contexts of agrochemical registration procedures with a particular emphasis on vertebrates, because for the European environmental risk assessment of agrochemicals species of birds, mammals and other vertebrates utilizing cropped fields, need to be known and monitored. We explored the effectiveness of active and passive air sampling devices. Both type of air samplers were placed in two different sites for 24h or 2 weeks, respectively. After the sampling, DNA was extracted from the filters using the DNA blood and tissue extraction kit. Afterwards, two different genetic markers (12S and 16S) targeting vertebrates, were amplified via polymerase chain reaction PCR. Following PCR-based amplification, amplicons were sequenced using the real-time portable nanopore sequencing. The sequencing data was analysed using the National Center for Biotechnology Information (NCBI) public genetic reference for the identification of present species available in the data base.

Our initial results are promising, showcasing potential for widespread application of eDNA sampling and analysis for species detection, monitoring and finally complete biodiversity surveys, and the use of these non-invasively and time-saving method in the context of species, crop or farmland community based environmental agrochemical assessments.

1.04.P-We020 Metabolomics Reveal Polyethylene Terephthalate Microplastic-Induced Metabolic Disturbances Dependent on Different Shapes and Sizes

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Microplastics (MP) are ubiquitously in aquatic environments. Their potential impacts and integration into the food web cause global concern. They are frequently found in marine organisms and environments with diverse shapes, sizes, and characteristics. In this study, we used the Mediterranean mussel *Mytilus galloprovincialis* as a marine benthic organism model to investigate the metabolic consequences of exposure to different polyethylene terephthalate MP sizes and shapes: round (27 32 μ m), small fibers (200 400 μ m), large fibers (3000 μ m), small fragments (20 μ m), medium fragments (45 75 μ m), and large fragments (>150 μ m). After exposure to high concentrations of MP for 14 days, round and small fiber-type MP were highly accumulated in mussels. Metabolomic analysis revealed that exposure to round and small fiber-type MP induced significant changes in 150 metabolites. Partial least squares-discriminate analysis (PLS-DA) showed that the round and small fiber MP treatment groups displayed similar cluster patterns that differed from those of the control group. In addition, only 22 annotated metabolites related to histidine, valine, leucine, and isoleucine degradation/biosynthesis and vitamin B6 and aminoacyl-tRNA biosynthesis were significantly affected by round or small fiber-type MP. Among the histidine metabolites, round and small fiber-type MP upregulated the levels of L-histidine, L-glutamate, carnosine, imidazole-4-acetaldehyde, 4-imidazolone-5-propanoate, and methylimidazole acetaldehyde and downregulated methylimidazole acetic acid and N-formimino-L-glutamate. These results suggest novel insights into the potential pathways through which MP of specific sizes and shapes affect metabolic processes in mussels.

1.04.P-We021 Transcriptomic Analysis of *Daphnia magna* Following Exposure to Biodegradable and Conventional Microplastics: Insights Into Molecular Toxicity Pathways

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Traditional toxicity assessments often reveal minimal phenotypic responses in *Daphnia magna* exposed to biodegradable or conventional microplastics, suggesting that endpoints such as growth and reproduction

may not fully capture the toxicity of these materials. To detect significant differences, studies often use unreasonably high microplastic concentrations, leading to conclusions that may not accurately reflect the actual impacts of microplastics on aquatic organisms. In contrast, transcriptomic analysis offers a highly sensitive approach to detecting gene expression changes caused by environmental pollutants, including microplastics. This study conducted a 21-day chronic exposure test on *D. magna* using polylactic acid (PLA) and polyethylene terephthalate (PET) microplastics. After the exposure period, RNA sequencing was performed to analyze transcriptomic changes. It is anticipated that distinct gene expression profiles will emerge across the control, biodegradable, and conventional microplastic exposure groups. These transcriptomic changes are expected to reveal both unique and overlapping molecular pathways affected by each type of microplastic. This study aims to provide valuable insights into the molecular mechanisms of microplastic toxicity and to identify potential biomarkers for exposure to biodegradable and conventional microplastics, offering a deeper understanding that cannot be achieved through phenotypic assessments alone. This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (NRF-RS202400335682)

1.04.P-We022 Size- and Shape-Dependent Toxicity of Microplastics on the Marine Rotifer *Brachionus koreanus*: In Vivo Toxicity and Multi-Omics Approach

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The marine microplastic (MP, < 5mm) pollution has become a major environmental issue worldwide. As the small particulate pollutants similar in size to prey, MPs can be readily consumed by aquatic organisms, including small zooplanktons, leading to various toxic effects. Previous studies suggest that the physical properties of MPs such as size and shape are important to determine their toxicity, but underlying mechanisms of size- and shape-dependent toxicity of MPs are not well-understood. Thus, in this study, we investigated multigenerational chronic toxicity of MPs of different sizes and shapes (fragment-S, 0.5- μ m; fragment-L, 5- μ m; bead-S, 0.74 to 4.99- μ m; bead-L, 3 to 16- μ m) to the marine rotifer *Brachionus koreanus*. Subsequently, to determine underlying toxic mechanisms of MPs, transcriptomic and metabolomic modulation in *B. koreanus* were analyzed following acute MP exposure. The chronic toxicity test showed that fragment-S significantly reduced fecundity of rotifers, which was enhanced by following generations (F1 and F2). In contrast, other MPs didn't affect reproduction of rotifers. Transcriptomic analysis showed that 187 and 151 number of genes were significantly modulated after exposure to 5 mg/L of fragment-S and fragment-L, respectively, whereas only 4 and 2 genes were significantly affected at the same concentration of bead-S and bead-L, respectively. Metabolic analysis also showed that the most significant metabolomic changes were observed in the group exposed to fragment-S (5 mg/L; 304 metabolites), followed by fragment-L (5 mg/L; 178 metabolites), but not in groups exposed to spherical MPs (less than 11 metabolites at the same concentration). The gene ontology (GO) and pathway analysis revealed that the major toxic mechanism of MPs is disruption of metabolic processes, such as metabolism of carbohydrate, lipid, and protein, and secretion of digestive enzymes, which was more significantly modulated after exposure to fragment-S. In addition, stress response and cell signaling pathways were mostly affected by fragmented MPs, especially fragmented-S, implying different toxic mechanisms of MPs depending on their size and shape. Our findings suggest that MPs can negatively affect population of marine biota through disturbing their energy metabolism, which can be enhanced with smaller and rougher MPs. This finding gives us an insight into the risk assessment of MP in marine environments. This research was supported by the Risk assessment to prepare standards for protecting marine ecosystem of the Korea Institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (KIMST-20220383).

1.04.P-We023 An Integrated Multi-Omics Approach to Uncover the Shape-Dependent Toxicity Mechanisms of Microplastics in Juvenile Rockfish *Sebastes schlegeli*

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Microplastics (MPs) of various sizes and shapes are formed through the degradation of plastic debris in marine environments. While continuous exposure to MPs is a concern, the impact of MP morphology on aquatic organisms remains relatively unexplored. This study investigated the toxic effects of two common MP shapes, fragments and fibers, on juvenile black rockfish (*Sebastes schlegeli*). Fish were exposed to concentrations of 0.2, 2, 20, and 400 mg/L of each MP type. Although no significant mortality was observed, exposure to both MP types induced cytotoxicity, as evidenced by alterations in apoptosis, phagocytosis, and cell cycle progression. Notably, fiber-shaped MPs exhibited greater toxicity compared to fragments. Transcriptomic analysis revealed differential gene expression patterns between the two MP

types. Exposure to fiber-shaped MPs resulted in a more pronounced downregulation of genes involved in immune response and cellular homeostasis. Metabolic analysis identified significant alterations in purine and arachidonic acid metabolism pathways. Our findings highlight the importance of considering MP morphology in assessing ecological risk. The observed shape-dependent toxicity of MPs can inform future pollution management strategies and environmental monitoring programs.

1.04.P-We024 Comparing In-Silico Predicted and Observed Transcriptomic Responses of *Gammarus pulex* to Micropollutants in Germany

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Chemical pollution is recognised as one of the major drivers influencing the ecological status of aquatic ecosystems. Micropollutants are organic chemicals occurring at trace environmental concentrations, including pesticides, pharmaceuticals, personal care products, and industrial chemicals among other, which have raised concern due to their negative impact on aquatic organisms. Some of these chemicals have known mode and mechanism of action (e.g., neonicotinoid insecticides altering nicotinic acetylcholine receptors). Nevertheless, for many chemicals their mode of action is unknown. Aquatic benthic invertebrate fauna represents one of the Biological Quality Elements, which is used to determine the Ecological status of surface water bodies according to the EU Water Framework Directive. They are therefore extensively used as biological indicators to assess water quality. Nevertheless, a thorough understanding of the adverse biological consequences of micropollutant mixtures at the pathway level under real exposure conditions is still absent. In this study, we used in-silico predictions to assess the interaction of micropollutants with proteins and subsequently enrichment pathways analyses were conducted to identify pathways of interest. In addition, we used RNA-based-sequencing (transcriptomic) approach on *Gammarus pulex* populations, shredder invertebrates, across a gradient of stations with increasing chemical pollution in the River Holtemme. Non-targeted gene assessment reveals alterations in gene expression, with an increasing number of differentially expressed genes (DEGs) downstream of chemical pollutant influences. Notably, a station downstream of a wastewater plant exhibits up to 6014 DEGs compared to the reference site. Our findings indicate a significant upregulation of invertebrate DEGs downstream of wastewater treatment plants. Furthermore, a pathways enrichment analysis identifies over-represented gene classes in categories such as nervous system development, carcinogenesis, and stress response across all stations under micropollutant stress. Thus, our results link observed biological responses to specific chemical groups, shedding light on the intricate interplay of micropollutants and their impact on freshwater macroinvertebrates in freshwater ecosystems.

1.04.P-We025 Temporal Trends in Transcriptomic Changes Induced by Suspended Particulate Matter Extracts in Zebrafish Embryos

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Over the years, organic contaminants have affected aquatic ecosystems such as rivers, often reaching concentrations that pose risks to exposed biota. In the context of assessing complex environmental samples, (higher throughput) screening methods are gaining importance as tools for research and potential regulatory application. The presented project aims to evaluate the integration of effect-based approaches, such as modified acute fish embryo toxicity test (FET) including transcriptomics, into environmental monitoring. As a proof of concept, the ecotoxicological potential in the river Rhine will be assessed by testing extracts of suspended particulate matter (SPM) samples from the Federal Environmental Specimen Bank (ESB) in the zebrafish embryo model, particularly investigating temporal and spatial trends. SPM samples from the Rhine at Weil and Koblenz from the years 2005 to 2021 were provided by the German ESB and extracted using the pressurized liquid extraction. In a range-finding FET with *Danio rerio*, lethal as well as a wide range of sublethal effects were observed in concentrations from 1.25 to 20 mgSEQ/mL. Embryotoxicity varied between both sites and throughout the time series with higher effects for Weil in the years 2005 and 2009 and for Koblenz in 2013 and 2017. For transcriptome analysis, embryos will be exposed to sublethal (10%-) effect concentrations. To identify potential chemical groups as drivers of toxicity, data will be correlated with existing chemical profiles of the SPM samples. Additionally, transcriptome results from the zebrafish will be compared to those from *Abramis brama* samples from the same years and locations to better understand the relevance of laboratory exposure experiments for real environmental scenarios and the relevance of screening methods for monitoring.

1.04.P-We026 Effects of Alternative Phthalates on Growth Hormone-Related Endocrine System, Neurobehavioral Development and Oxidative Stress in Zebrafish Larvae

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Although there is growing concern about the presence of alternative phthalates in the environment, knowledge about their potential toxicity is quite limited. In this study, we evaluated the growth hormone (GH)/insulin-like growth factors (IGFs)-related endocrine system, neurobehavioral development, and oxidative stress induction of acetyl tributyl citrate (ATBC), acetyl triethyl citrate (ATEC), dibutyl adipate (DBA), and di-(2-ethylhexyl) adipate (DEHA) using zebrafish embryo/larvae. Zebrafish embryos were exposed to ATBC, ATEC, DBA, and DEHA (0, 0.03, 0.3, 3, 30, and 300 µg/L) for 96 h, and changes in developmental parameters, behavior, hormones and gene transcriptions related to GH/IGFs axis, reactive oxygen species, and antioxidant enzymes were measured. ATBC exposure reduced body length and moving distance of larvae, while no significant effects on development and behavior were observed in larvae exposed to ATEC, DBA, and DEHA. Hypoactivity, decreased levels of GH, IGF-1, and acetylcholinesterase activity, and down-regulation of genes involved in central nervous system were observed in fish exposed to ATBC. The levels of superoxide dismutase, catalase, and glutathione peroxidase were significantly increased in fish exposed to ATBC. Pretreatment with N-acetylcysteine alleviated ATBC-induced GH-related endocrine disruption and neurobehavior toxicity. The results of this study revealed that behavior impairments and developmental delay induced by ATBC exposure are attributed to oxidative stress. This study was supported by the National Research Foundation of Korea (NRF; Project no. RS-2023-00251751).

1.04.P-We027 Investigation of Adverse Outcome Pathways Associated with Preeclampsia and Use of Zebrafish to Screen Environmental Pollutants

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Preeclampsia is a pregnancy disorder characterized by high blood pressure and proteinuria. Preeclampsia can lead to severe complications for both mothers and babies, including placental abruption and stillbirth. The heritability is estimated to be approximately 55%, and contributing factors include maternal nutritional deficiencies and exposure to environmental pollutants such as cadmium, bisphenol A, and phthalates, etc. Understanding the important causes of preeclampsia is essential to identify risks and screening chemicals associated with its development. Although zebrafish lack a placenta, they express genes such as vegfaa, sflt-1, eng, and hif1aa, which are orthologous to human genes involved in preeclampsia. Zebrafish shared some of their molecular mechanisms with humans, making them a useful model to study preeclampsia. In this study, adverse outcome pathways (AOPs) were investigated to identify molecular initiating events (MIEs) and key events (KEs) relevant to preeclampsia. Important angiogenic markers, including vascular endothelial growth factor (VEGF), soluble fms-like tyrosine kinase-1 (sFLT-1), soluble endoglin (sEng), and placental growth factor (PlGF), were evaluated along with pathways associated with exposure to environmental pollutants. An AOP tree was constructed to compare preeclampsia-related mechanisms between humans and zebrafish. The renin-angiotensin-aldosterone system (RAAS) pathway was selected for experimental validation. Zebrafish embryos were exposed to vatalanib, a VEGF receptor inhibitor, at concentrations of 0, 40, 200, and 1000 µg/L for 96 h. VEGF protein levels were measured using enzyme-linked immunosorbent assay, and gene expression for vegfaa, sflt-1, eng, and hif1aa was analyzed using quantitative real-time polymerase chain reaction. Proteinuria was assessed using BCA protein assay kits. Vatalanib exposure altered VEGF levels and the expression of angiogenesis-related genes, indicating potential impacts on angiogenic pathways. Results indicate that zebrafish angiogenic biomarkers, including VEGF, vegfaa, sflt-1, hif1aa, and eng, are valuable for studying preeclampsia mechanisms. These findings suggest the potential of zebrafish as a predictive model for identifying chemicals linked to preeclampsia. Further studies are needed to investigate the effects of various environmental pollutants on the angiogenic pathway in zebrafish. This work was supported by Korea Environmental Industry & Technology Institute (KEITI) through Core Technology Development Project for Environmental Diseases Prevention and Management, funded by Korea Ministry of Environment (MOE) (RS-2022-KE002173)

1.04.P-We028 Transcriptomic Responses of Embryonic Flounder (*Paralichthys olivaceus*) to Hull In-Water Cleaning Wastewater Exposure

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Antifouling paints, which contain biocidal compounds, are applied to boat and ship hulls to prevent or minimize the attachment of fouling organisms. Despite the potential toxicity risk associated with these pollutants, there is a limited number of studies investigating and monitoring the toxic effects on embryonic

fish using hull in-water cleaning (IWC) wastewater collected from ship. In this study, hull IWC wastewater from ship was collected from a ship in 2022 and toxic effect assessments were conducted on fertilized embryonic Olive flounder (*Paralichthys olivaceus*). After dividing the IWC wastewater into untreated wastewater and filtered wastewater, fertilized embryos were exposed to various dilution factors (10-, 100-, and 1000-fold dilutions). Chemical analysis of the IWC wastewater revealed high proportions of Cu, Fe, and Zn. There was no significant difference in the mortality of embryonic flounder exposed to untreated wastewater compared to filtered wastewater. However, malformations in morphogenesis, including pericardial edema, dorsal curvature, tail fin fold defects, and developmental delays, were observed in fertilized embryos following exposure to IWC from ship. To understand the molecular biology of malformation, eight genes related to (heart formation (nkx2.5, NK2 NK2 homeobox 5; SOX6, SRY-box containing gene 6; robo1, roundabout receptor1), bone malformation (bmp4, bone morphogenetic protein 4), fin malformation (plod2, procollagen-lysine 2-oxo-glutarate 5-dioxygenase 2, furin, furin, paired basic amino acid cleaving enzyme; wnt3a, Wnt family member 3a), and tumors (TP73, tumor protein p73) were evaluated using qRT-PCR. To clarify the potential toxic effects of IWC wastewater, we also conducted RNA-seq (high-throughput sequencing) on embryonic flounder exposed to hull IWC wastewater. In embryonic flounder exposed to IWC wastewater, genes related to nervous system development, cell development, muscle development, and animal organ development pathways were significantly differentially expressed. This study provided crucial evidence of the risks associated with IWC wastewater when exposed to marine organisms. Taken together, these results may inform strategies to improve hull-cleaning wastewater pollution management to better protect coastal ecosystems.

1.04.P-We029 Analyzing the Metabolic Effects of Nitrobenzene Exposure in Japanese Medaka (*Oryzias latipes*) with Consideration of Reproductive Cycle

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For impact investigation of chemical accidents in Korea, National Institute of Chemical Safety suggest the Criteria for calculating damage scale. Among them, in the case of biological resources, damage caused by chemical is recognized when death, disease, abnormal behavior, physical dysfunction, and external deformation occur. However, based on the current standards, the damage can be recognized only visually identifiable damaged fish, determining damage based on abnormal behavior or alteration of appearance is subjective and unclear. As a result, there is no standard for assessing the damage to fish that were near the accident site but did not die, even though they may have been affected by chemicals invisibly. Therefore, it is crucial to establish accurate standards for distinguishing fish damaged by chemicals. We chose nitrobenzene as an experimental chemical, because it is a highly toxic chemical to fish and showing a lot of usage in Korea. Therefore, this research investigates the impact of nitrobenzene on fish metabolism, particularly in Japanese medaka (*Oryzias latipes*), considering their reproductive cycle. We selected a sampling method to acquire samples from both the control and experimental groups as a singular entity, involving the concentration and measurement of extracorporeal metabolites released into the media over a designated time interval. We sampled four times a day in accordance to verify metabolic changes, which are characterized by daily ovulation. In this study, an investigation of the impact of both the reproductive cycle and nitrobenzene exposure on the extracorporeal metabolites of medaka was undertaken. distinctive identification of metabolites that exhibited a notable decrease post nitrobenzene exposure, as compared to their pre-exposure state, indicated an encompassing influence on the reproductive cycle. In further, enumeration and annotation of metabolites displaying significant changes in each analysis will be conducted. Subsequently, we will identify biomarkers to differentiate changes attributed to the reproductive cycle and those induced by nitrobenzene exposure. This work was supported by Korea Environment Industry & Technology Institute (KEITI) through Advanced Technology Development Project for Predicting and Preventing Chemical Accidents Project, funded by Korea Ministry of Environment (MOE) (2022003620001).

1.04.P-We030 Investigating the Effects of N-alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) on the Fathead Minnow (*Pimephales promelas*) Proteome, in a Two-Phase Approach and as Part of a Whole-Ecosystem Study

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N-alkyl dimethyl benzyl ammonium chloride (ADBAC) is a chemical disinfectant often integrated into household personal care products, surface cleaners, and cosmetics. Consumer ADBAC usage has significantly increased due to the ongoing COVID-19 pandemic. Individuals discharge these chemicals

into wastewater, where the contaminants persist and become a threat to aquatic organisms. Although researchers have identified ADBACs in environmental water samples at ug/L concentrations, few studies have examined their toxicological effects on fish. When fish are exposed to contaminants, they may develop molecular changes within their tissues and biofluids. Scientists can utilize proteomics to determine the effects of contaminants on protein abundance, and they can examine potential protein biomarkers that are tied to adverse outcome pathways. We aimed to utilize a proteomic approach to determine the effects of an ADBAC mixture on the fathead minnow (*Pimephales promelas*). We exposed 330 fathead minnows to 4 concentrations of a benzalkonium chloride mixture (0.02ug/L, 0.2ug/L, 2ug/L, and 20ug/L) and solvent control (0ug/L) over a 28-day period. Following the ADBAC exposure, we anaesthetized the fish with 100 mg/L pH buffered tricaine methanesulfonate. Then, we collected mucus, plasma, and tissue samples. Next, proteins were sequenced with liquid chromatography-tandem mass spectrometry. Simultaneously, the International Institute of Sustainable Development Experimental Lakes Area (IISD-ELA) is conducting a multi-year, whole-lake ADBAC exposure. In this presentation, we will share the methodology from both phases of the study. Additionally, we will highlight the preliminary plasma and gill proteomics results from the laboratory exposure.

1.04.P-We031 Chronic Pollutant Exposure: Implications for Estuarine Fish Under Multi-Stressor Conditions

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Estuaries are subject to a range of environmental and anthropogenic stressors, particularly pollutants, which pose significant risks to aquatic life. Current research on the impacts of pollution and associated dose-response relationships in aquatic organisms tends to focus on single-treatment experiments in controlled laboratory settings with relatively short exposure durations. However, real-world estuarine environments often involve chronic exposure to complex mixtures of pollutants, exacerbated by additional stressors such as rising temperatures and hypoxia. These factors may interact in ways that are either synergistic or antagonistic, complicating the understanding of their cumulative effects. This study aims to investigate the impact of chronic exposure to micropollutants on two fish species within the Elbe estuary. Employing transcriptomic analyses, comet assays, and tissue analysis, samples were collected from five stations along the estuarine gradient across three seasons. The study builds upon a xenobiotic stress response observed in a previously conducted extensive transcriptomic data set. Given that molecular stress responses to pollution are known to intersect with pathways activated by hypoxia and elevated temperatures, the hypothesis is that chronic exposure to micropollutants may compromise the resilience of estuarine fish populations to climate change. This investigation aims to enhance understanding of how environmental pollutants impact aquatic organisms in the context of multi-stressor scenarios. This research is conducted in the research training group 2530 funded by the German Research Foundation.

1.04.P-We032 Optimization of Non-Lethal Fish Epidermal Mucus Collection Methods for Remote Fieldwork, Community-Based Monitoring, and Community Science

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Fish epidermal mucus is one of the first lines of defence against chemical, physical and biological stressors. Acting as a natural, semi-permeable, chemical and biological barrier, fish epidermal mucus plays a critical role in the animal's immunological and protective function due to its mechanical and biochemical properties. As such, fish epidermal mucus is a repository of numerous innate and acquired components of immunity. Fish epidermal mucus is produced and sloughed off continuously, allowing for its non-invasive collection with relative ease compared to other methods (i.e., blood and tissue sampling). Mucus collection methods can be conducted with relative ease in a laboratory setting, but collection, handling, and preservation methods need to be developed and optimized for remote field sampling. The goal of this study was to optimize current fish epidermal mucus collection methods for remote field settings by testing if we could collect epidermal mucus on paper wipes (i.e., Kimwipes) and allowing them to air-dry, thereby eliminating the need for immediate laboratory interventions (i.e., reagent addition, centrifugal filtering, and cryopreservation). Simplified methods enable laypersons without a science background to easily collect samples, allowing for longer-term, community-based monitoring programs that prioritize humane, non-lethal, and minimally invasive methods, advancing field-based research scopes and opportunities beyond what was previously possible. In a laboratory setting, we collected epidermal mucus samples from sedated rainbow trout (*Oncorhynchus mykiss*) and lake sturgeon (*Acipenser fulvescens*) and stored samples in different conditions (i.e., aluminum foil sheets and plastic bags) and durations (i.e., 10, 20, and 30 days). We then measured proteins in epidermal mucus using liquid chromatography and non-targeted tandem high-resolution mass spectrometry paired with

chemoinformatics and bioinformatics software. We present preliminary results from this study and discuss the next steps of our research.

1.04.P-We033 Exploring the Trophic Transfer Risks of Titanium Dioxide Nanoparticles in Aquatic Organisms

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Titanium dioxide nanoparticles (TiO₂ NPs) are widely used nanomaterials, commonly found in consumer products and cosmetics, such as sunscreens. Despite being regarded as biologically "safe" due to their inability to penetrate the skin, TiO₂ NPs can enter aquatic ecosystems through wastewater discharge or recreational activities like swimming. Consequently, titanium and TiO₂ NPs have been detected in aquatic environments. While partially excreted after ingestion, both titanium and TiO₂ NPs can accumulate in fish livers, where their presence has been associated with hepatocyte ultrastructural alterations, metabolic disturbances, inflammation, oxidative stress, and DNA damage. Since these effects depend on the route of exposure, this study investigates the impacts of 25 nm TiO₂ NPs across an aquatic trophic transfer chain comprising microalgae (*Oxyrrhis marina*), brine shrimp (*Artemia salina*), and zebrafish (*Danio rerio*). Preliminary findings indicate that *O. marina*, a highly phagocytic microalga, functions as a trophic vector for TiO₂ NPs. Further studies with *A. salina* revealed developmental and pigmentation alterations, as well as mobility impairments, upon consuming algae preloaded with TiO₂ NPs. Behavioural changes, nutritional composition, and metabolic biomarkers in *A. salina* are still being evaluated. The study extends to *D. rerio* exposed to TiO₂ NPs via *A. salina*, with comparisons being made between TiO₂ NPs ingested via contaminated water or via microalgae. Distinct mechanistic pathways, molecular targets, and biological roles seem to be associated with these two different exposure routes. Therefore, omic approaches (proteomics, lipidomics, microbiomics) and other methodologies are being applied to assess and link metabolic alterations, nutritional impacts, gut microbiota changes, and titanium accumulation in zebrafish. By examining these cascading effects, this research aims to enhance our understanding of TiO₂ NP behaviour in aquatic food webs, offering critical insights for environmental risk assessment and ecological management. This work was supported by the Portuguese Foundation for the Science and Technology (FCT) through the project NanoPlanet 2022.02340.PTDC, and through UIDB/04423/2020 and UIDP/04423/2020 contracts. M.J.A. and A.C. also acknowledge the FCT funding for the Scientific Employment Stimulus Program (2023.06491.CEECIND and CEECIND/03767/2018, respectively).

1.04.P-We034 Toxicokinetics of Perfluorinated Compounds (PFCs) in Frogs (*Rana tigrina cantor*) Via Skin Exposure and Effects of Hibernation

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We investigated the bioaccumulation and effects of hibernation on the toxicokinetics and maternal transfer of perfluorinated compounds (PFCs) in frogs via skin exposure. The assimilation efficiencies (AE) ranged from 9.4–84% and were negatively correlated with the carbon chain length of short perfluorocarboxylic acids (PFCAs) (<9) but positively correlated with perfluorosulfonic acids (PFSAs). The AE pattern for long-chain PFCAs was found to be similar to that for PFCAs in hens. The egg was the main site for the deposition of PFSAs and long PFCAs, whereas the carcass was the main organ for short PFCAs. The maternal transfer of PFCs increases over time, reaching more than 80% of the total body burden for some compounds. No clear trend was observed between the egg-maternal ratio (EMR) and the carbon chain length of the PFSAs. For PFCAs, the EMR first sharply increased and then decreased with carbon chain length, reaching a maximum at an approximate carbon chain length of 10. Hibernation inhibited the development of eggs in frogs and resulted in bioamplification factors (BaMFs) of 1.79 and 2.70 for total PFSAs and PFCAs, respectively. BaMFs for the liver (5.3–16.2) were larger than BaMFs for other tissues or organs, indicating hibernation significantly raised the concentrations of PFCs in the liver. The relative enrichment of PFCs in eggs decreased during hibernation. The bioamplification of PFCs in maternal tissues or organs is mainly caused by a decreased biotransformation rate of PFCs, whereas in eggs, it is mainly caused by weight loss. The results of the present study are significant for understanding the bioaccumulation and toxic effects of PFCs in oviparous animals with hibernation of physiological processes.

1.04.P-We035 Hepatic Transcriptome Alterations of *Xenopus Laevis* Following Multi Route Exposure to Graphene Oxide

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Graphene-based nanomaterials, such as graphene oxide (GO), exhibit unique properties that have driven their widespread use in industrial and medical applications. However, the potential release of GO into aquatic ecosystems throughout its life cycle raises the need for comprehensive ecotoxicological risk assessments to ensure its safe use. Amphibians, highly sensitive to environmental contaminants, have been studied for various toxic effects of GO, including genotoxicity, primarily at the larval stage through direct exposure. This study expands the scope by investigating the combined effects of direct and trophic GO exposure on post-metamorphic *Xenopus laevis*, focusing on hepatic transcriptomic analysis to explore GO's mechanisms of action on a critical organ involved in metabolism, erythropoiesis, and detoxification. To simulate realistic exposure scenarios, dipteran larvae were exposed to varying GO concentrations for 24 hours before being introduced as prey for juvenile *Xenopus*. The amphibians consumed GO-laden larvae while simultaneously being exposed to GO in the water column. mRNA was extracted from the whole liver of *Xenopus* after 24 and 96 hours and sequenced using high-throughput technologies to investigate transcriptomic profiles and identify GO-affected biological pathways. Differential gene expression analyses, coupled with gene ontology functional annotations, revealed transcriptomic disruptions induced by GO at concentrations as low as 0.1 mg/L, which are environmentally relevant. After 96 hours of exposure, the results suggested that GO primarily affects lipid and sugar metabolism as well as cell cycle regulation mechanisms. This was further confirmed by flow cytometry analysis of the cell cycle in erythrocytes, which showed an increase in mitotic cells, potentially indicating compensatory mechanisms in response to hypoxic conditions caused by GO. This study highlights multiple pathways of disruption in organisms exposed to GO under realistic conditions. The findings emphasize the need for long-term and multi-generational studies to fully assess the ecological impact of these nanomaterials and avoid underestimating their effects in aquatic ecosystems.

1.04.P-We036 Protein Panel to Assess Toxicity Mechanisms and Effects in the Zebrafish PAC2 Cell Line

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Fish cell-based assays provide valuable animal-free alternatives for predicting chemical toxicity to fish. While these in vitro assays are already well-established for the assessment of acute effects on cell viability, gaining a deeper understanding of molecular mechanisms of toxicity could enhance their predictive power in the context of chronic toxicity. Omics methods can deliver large-scale overviews of toxicant-induced molecular alterations. So far, transcriptomics has been applied most often, but protein-level studies could offer more substantiated insights into different cellular responses and their consequences, as proteins are more closely linked to phenotype. Using mass spectrometry-based targeted proteomics which allows measuring multiple proteins in one sample simultaneously, protein panels for toxicity assessment can be established, similarly to gene-based panels such as the S1500 list. Here, we present a tentative protein marker panel for monitoring toxicity effects and outcomes in cell lines of zebrafish (*Danio rerio*), chosen for the pilot studies because it is a well-studied fish model species with sequenced genome and substantial molecular resources available. To select candidate proteins for the panel, we performed a narrative review of studies reporting on genes and proteins associated with major mechanisms and effects of toxicity on molecular and cellular levels. For selected genes, 220 corresponding zebrafish proteins were identified in the UniProt database. While a large proportion of the pathways covered in the resulting list are related to cellular stress responses, as expected, also several other pathways, including lipid metabolism, longevity regulation and immune responses emerged as potentially important for toxicity responses. Candidate protein markers will be tested in the zebrafish PAC2 cell line exposed to a set of reference chemicals, including, e.g., 2,4-dinitrophenol (a protonophore disrupting cellular ATP synthesis), diethyl maleate (inducer of oxidative stress), nocodazole (inhibitor of microtubule polymerization), tunicamycin (inducer of endoplasmic reticulum stress via inhibition of protein glycosylation), and thapsigargin (inducer of endoplasmic reticulum stress via increase of cytosolic calcium ion levels). This work contributes to the establishment of a novel toxicity assessment method that relies on a simultaneous measurement of multiple protein markers to monitor toxicity mechanisms and effects in fish models. This research is funded by the Swiss National Science Foundation through National Research Programme 79 Advancing 3R Animals, research and society, project number 407940_206439, Expanding the fish invitrome towards a modular, socio-technical framework for animal-free prediction of chemical toxicity to fish.

1.04.P-We037 Time-Resolved Analysis of Trifloxystrobin-Induced Proteome Alterations in a Fish Cell Line

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Trifloxystrobin is an organofluorine fungicide that has a broad application in agriculture. This substance disrupts cellular energy production cycle through effects on mitochondrial respiration, where it binds the ubiquinone binding site (Qo) of cytochrome b, leading to the obstruction of the flow of electrons between cytochrome b and cytochrome c1. While having lower toxicity in terrestrial animals, such as birds, trifloxystrobin shows high toxicity to aquatic organisms such as fish. In this study, we investigated the effects of trifloxystrobin exposure on the proteome of zebrafish (*Danio rerio*) PAC2 cells, aiming to identify the disrupted molecular pathways and evaluate the responses across the time continuum from acute (several hours) to chronic (several days, covering the doubling time period for this cell line). Cells exposed to trifloxystrobin at the concentration known to cause 50% reduction in cell population growth were harvested at 4, 24, 48 and 120 h post exposure and processed for global proteomics analysis using the S-trap and trypsin digestion. Peptides were analyzed by nanoLC-MS/MS in data-independent acquisition (DIA) mode, followed by directDIA library-free data processing workflow with Spectronaut (Biognosys) for peptide identification and protein group assignment, followed by post-processing analysis on the Perseus platform (MaxQuant). Proteomics analysis revealed the existence of several highly correlated clusters of proteins that tended to be either up or downregulated in response to trifloxystrobin exposure. In some clusters, stronger effects were observed in the beginning of exposure but disappeared with increasing exposure time. In other clusters, increasing exposure time led to stronger effects on protein expression. Differentially expressed proteins are now being investigated for their involvement in specific molecular pathways disrupted by trifloxystrobin exposure, as well as in relation to their suitability as biomarkers of long-term exposure to trifloxystrobin or similar compounds in the environment. We also aim to evaluate the similarity of omics responses observed *in vitro* with results of *in vivo* studies reported in the literature. This work provides a broad overview of proteome alterations induced by trifloxystrobin exposure in fish cells and could allow identification of protein biomarkers for follow up studies in other cell lines, fish embryos, or environmental samples such as fish tissues collected in the wild.

1.04.P-We038 Assessing the Effects of Emerging Contaminants on Gene Expression in *Stenella coeruleoalba* Biopsies and Human Cell Cultures

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In recent years, new classes of pollutants have received the attention of scientists and regulators. Many of these compounds, defined as contaminants of emerging concern (CECs), have not been well studied, making predictions of their toxicity on biota. CECs enter the environment from various anthropogenic sources and are distributed throughout environmental matrices. They have been detected in the marine environment at concentrations significantly higher than expected and their risk to human and environmental health may not be fully understood yet. CECs include a vast array of contaminants: such as pharmaceuticals and personal care products (PPCPs); plastic additives such as bisphenol A (BPA) and phthalate esters (PAEs) which are both commonly spread in marine organisms ranging from invertebrates up to top predators. There are currently few studies in literature regarding the impact of emerging contaminants towards organisms at highest level of the trophic chain, such as marine mammals, and also humans. Further studies are needed to better understand the toxicological risk that these contaminants may pose to the marine ecosystem and human health. We identified by literature research and *in silico* analysis a set of molecular endpoints involved in the homeostasis of the organism to better understand the effect that CECs may exert on mammals. By droplet digital PCR (ddPCR) we analyzed striped dolphin (*Stenella coeruleoalba*) skin biopsies and *Homo sapiens* cell cultures (fibroblasts, hepatocytes and CaCo-2), treated with mixtures of the most common and prevalent emerging contaminants, at realistic environmental concentrations (Ctrl, 1 µg/l, 10 µg/l, 100 µg/l), based on previously conducted environmental samplings. *Stenella coeruleoalba* biopsies and *Homo sapiens* cell cultures were treated with the following mixtures: a mix of pharmaceuticals consisting of Carbamazepine, Valsartan and Ibuprofen; a mix of phthalates consisting of 9 of the most prevalent phthalates and a super mix consisting of a mix of pharmaceutical, phthalates and bisphenol A. To better understand the effects of emerging contaminants in top predators, this study evaluated the alterations in gene expression profiles for the same molecular endpoints across both species. Through the assessment of these endpoints, it was possible to identify which contaminants that warrant increased attention due to their relevant effects on gene expression in top predators at environmentally relevant concentrations.

1.04.P-We039 Transcriptomic Analysis of Diesel Particulate Matter-Induced Molecular Effects in H9C2 Cardiomyocytes

Kyoung Jin Nho, Jae Hoon Shin and Jin Ee Baek, *Institute of Occupation and Environment, COMWEL, Korea, Republic of*

Diesel particulate matter (DPM) exposure is linked to adverse cardiovascular and respiratory health effects, driven by oxidative stress, inflammation, and apoptosis. This study examines the molecular impacts of DPM on H9C2 cardiomyocytes through an integrative analysis combining RNA sequencing data from DPM-exposed cells, publicly available GEO datasets (GSE47495, GSE9694), and findings from recent literature. Transcriptomic profiling revealed significant upregulation of genes associated with oxidative stress (e.g., SOD1, CAT), inflammation (e.g., TNF- α , IL-6), and cell death pathways. These findings align with shared molecular responses observed in the GEO datasets and literature, highlighting conserved mechanisms underlying DPM-induced cytotoxicity. Pathway enrichment analysis further supports the activation of antioxidant defense and inflammatory signaling as key drivers of cardiotoxicity. This integrative approach underscores the systemic impact of DPM exposure and the need for targeted protective strategies to mitigate its harmful effects on cardiovascular health.

1.04.P-We040 Impact of Neuroactive Pharmaceuticals and Pesticides on Excitability of Neuronal SH-SY5Y Cells: Role in Identification of Biomarkers of Effect of Neuroactive Compounds

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Rising levels of neuroactive compounds (NC) in the aquatic environment pose a novel risk to ecosystems. The promising strategy for environmental monitoring of NC would require development of bioanalytical tools including biomarkers of effect sensitive to NC exposure. Despite their key role in neuronal excitability, voltage gated channels are often overlooked in ecotoxicological studies, and their potential as biomarkers of effect of NC should be explored. This study investigated the effects of neuroactive pharmaceuticals and pesticides with different primary modes of action on the expression of genes encoding subunits of voltage-gated sodium (SCN4B and SCN1A) and calcium channels (CACNA2D1) in retinoic acid-differentiated SH-SY5Y cells. The cells were treated with 4 pharmaceuticals (sertraline, clozapine, carbamazepine, caffeine) and 4 pesticides (fipronil, imidacloprid, deltamethrin, diazinon) in the concentrations of 10 or 1 mg/L, 10 μ g/L and 10 ng/L, followed by RT-PCR analysis. The results revealed significant downregulation (up to 3.8 times) of SCN1A by caffeine, clozapine and sertraline at multiple concentrations. The SCN4B expression was also significantly inhibited by sertraline and caffeine at 10 ng/L (6.3 times and 2.7 times, respectively). Similarly, SCN1A expression was significantly inhibited by all tested pesticides (up to 5 times, in 10 μ g/L diazinon treatment) and SCN4B by fipronil, diazinon and deltamethrin, at specific concentrations. However, fipronil exposure at 10 μ g/L induced overexpression of voltage-gated sodium channel subunits. The expression of CACNA2D1 was also affected, with significant inhibition (up to 2.7 times) by caffeine, fipronil and deltamethrin, and stimulation (up to 2.2 times) by clozapine and carbamazepine, at specific concentrations. These findings demonstrate that subunits of voltage-gated sodium and calcium channels are sensitive to NC, suggesting potential adverse effects on neuronal excitability regardless of their primary mode of action. The observed changes, especially in SCN1A expression, highlight their potential as promising novel biomarkers of effect of NC. The research was supported by Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-66/2024-03/200125 and 451-03-65/2024-03/200125) and Science Fund of the Republic of Serbia, Program for Excellent Projects of Young Researchers (PROMIS), Project BIANCO No. 6061817.

1.04.P-We041 In Vitro Transcriptomic Analyses and MicroFlow Assay for Natural Anthraquinone Dyes

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Colourants are important components in all commercial and industrial products, such as food, textiles, cosmetics, and packaging materials. Synthetic dyes derived from fossil sources are massively used in the textile industry. These dyes may cause health risks to workers, end-users and to the environment (especially through water contamination). There is increasing interest in developing safer and more sustainable dyes. Natural anthraquinone dyes extracted from bloodred webcap fungus (*Cortinarius sanguineus*), such as dermocybin, dermorubin and emodin, are potential alternatives to synthetic dyes. However, natural origin per se does not mean that these biocolourants are non-toxic or safe to use. It is

known that emodin is mutagenic in *Salmonella* bacteria, while dermocybin and dermorubin are not mutagenic in this system. However, all of these anthraquinones increase reactive oxygen species production in human cell lines (Herrala et al., 2022, Yli-Öyrä et al., 2024). Therefore, we investigated the genotoxicity of these natural anthraquinone dyes using metabolically competent HepaRG cells to more closely mimic human liver biology. We used the MicroFlow assay to quantify micronucleus frequency (chromosome damage) and relative cell survival. To advance mechanistic understanding, transcriptomic profiles were produced using TempO-Seq (Templated Oligo assay with Sequencing readout) to analyze two biomarkers of DNA damage (TGx-DDI and GENOMARK). Ingenuity Pathway Analysis (IPA) was applied to identify upstream regulator of the altered genes and enriched canonical pathways. None of the dyes increased micronucleus formation and only emodin caused cytotoxicity. Interestingly, all three anthraquinone dyes resulted in extensive transcriptional perturbations, indicating some form of bioactivity, but they were non-genotoxic in biomarker analyses, in-line with the negative microflow results. No upstream regulators specifically explained changes in gene expression levels, but several canonical pathways were activated, mostly related to metabolism. In conclusion, dermocybin and dermorubin may provide suitable alternatives for synthetic dyes as they were not genotoxic in our models. Potent mutagenicity of emodin in bacteria did not translate to genotoxicity in human liver cells. However, transcriptomics revealed effects on gene expression levels that require further analysis to inform their implications to human health hazards. The research has been funded by the Research Council of Finland (grant #346744) and the UEF Water research programme, which is jointly funded by Saastamoinen Foundation, Jenny and Antti Wihuri Foundation, and Olvi Foundation.

1.05 Microbiota Under Stress: Impacts of Environmental Pollutants on Ecosystem and Host Health

1.05.T-01 Linking Pesticide Degradation with Microbial Community Shifts in Agricultural Soils

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While essential for modern conventional agriculture, pesticides can cause damage to soil microbial ecosystems, compromising both soil health and resilience. Some of the negative effects of pesticide application include, but are not limited to, decline in microbial diversity, degradation of soil structure, decrease in water holding and nutrient cycling capacity. The aim of this study is to investigate how pesticide application influences soil microbial community dynamics. We also study the role of soil texture, organic matter content and initial microbial community in determining pesticide purification capacity of soils.

We hypothesize that (1) soils with higher organic matter will have elevated microbial activity, enhancing pesticide degradation; (2) pesticide application will reduce microbial diversity and abundance; and (3) by integrating pesticide degradation kinetics data and microbial community molecular data across different soils, we will be able to identify specific microbial and fungal taxa shifts in response to pesticide breakdown. To test these hypotheses, we analyse three agricultural soils treated with a mixture of 11 pesticides, monitoring degradation kinetics alongside microbial shifts using RNA and DNA extractions and amplicon sequencing. Results are expected to highlight key microbial and fungal taxa involved in pesticide breakdown and reveal how initial soil characteristics modulate degradation efficiency. The findings of this study will provide valuable insights that can be utilized in bioremediation efforts, as they can inform targeted strategies to stimulate microbial and fungal communities and optimize amendment selection for sustainable agricultural soil management. Furthermore, deeper understanding of microbial community shifts in response to pesticide application may lead to development of novel microbiome-based soil health indicators. This research underscores the role of microbial communities in soil ecosystem functions, promoting more sustainable soil management and enhancing resilience to agricultural inputs for long-term productivity.

1.05.T-02 Investigating Microbiota and Geochemical Characteristics of Colorado Mining-Influenced Waters

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Acid-mine drainage (AMD) is a global environmental challenge that threatens natural ecosystems and human water supplies. The extreme conditions created by low pH and high metal concentrations make AMD ideal for characterizing unique extremophilic microorganisms which play several roles from cycling nutrients to perpetuating acid generation. The core microbiome of mining-influenced waters is well understood, but additional investigation of the rare biosphere is needed. A better understanding of how microbial community compositions vary with water chemistry in AMD can improve methods in

biomining, bioremediation, and mineral exploration and provide insights into ecosystem health. Our goal was to characterize the geochemistry and microbiology of 13 mining-impacted waters in the Colorado Mineral Belt and explore relationships between microbial community composition and water chemistry. Total and dissolved ion concentration, geochemical parameters (e.g. pH, DO, etc.), and 16S/18S rRNA genetic sequences were measured for each location. Due to high loads and Colorado's underlying geology, rare earth elements (REEs) were of particular interest. In our samples pH was a master variable controlling concentration and dissolution of several metals including total REEs. REEs were also correlated to dissolved oxygen, aluminum, manganese, copper, zinc, cadmium, chromium, and nickel. AMD microbial communities commonly include *Leptospirillum*, *Ferroplasma*, and *Acidothiobacillus* and we expect to find these genera in the core microbiome of all or most of our samples. We hypothesize that the rare biosphere will contain community members or compositions unique to the water chemistry of that sample and will specifically analyze possible relationships between the presence of different taxa, REEs, and other correlated variables mentioned previously. This investigation provides a simultaneous assessment of the geochemical and microbial composition of mining influenced waters over a large geographical range. Exploring relationships between microbial community compositions and water chemistry and geology in MIWs provide the potential opportunity to better understand novel extremophilic taxa. Relationships between the presence or abundance of specific genera and water chemistries will be presented with a particular focus on relationships with total REEs.

1.05.T-03 Assessing Microbial Community Functions as an Indicator of Marine Ecosystem Disturbance

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Microbial Ecotoxicology aims to study (i) the impact of chemical and/or biological pollution on microbial communities and their ecosystem functions, and (ii) their role in pollutant ecodynamics. Microbial communities are valuable indicators of ecosystem health and recovery due to their rapid responses to pollutants but only a limited number of studies provide information on their functional properties essential for maintaining key ecosystem resilience. Therefore, metagenomic approaches, combined with other analyses such as metabarcoding, could provide insights into functional diversity to address this issue. Here, we investigated the potential of metagenomic analysis alongside molecular fingerprinting to evaluate the functional resilience of microbial communities to an anthropic poison using 6 concentrations of 3,5 dichlorophenol after 1 hour, 7, 15, 20, and 42 days of exposition. Metagenomic, metabarcoding and microbial analyses revealed time- and dose-dependent effects of 3,5-dichlorophenol on marine microbial communities and functions. Exposure to increasing doses of 3,5-dichlorophenol showed no significant impact on microbial functions over 42 days at concentrations up to 210 µg.L⁻¹ (1X, known NOEC for standard tests on *Daphnia*). However, at 2100 µg.L⁻¹, microbial functions gradually shifted, with changes in bacterial communities and activation of metabolic pathways related to organic compound degradation, indicating pollutant biodegradation. At 21 mg.L⁻¹ (10X), rapid shifts in microbial functions and communities suggested short-term lethality followed by activation of protective metabolic pathways. Although xenobiotic degradation pathways were triggered, the high dose exceeded biological capacity. These findings confirm the value of metagenomic analyses for assessing the impact of pollutant on microbial biodiversity functions.

1.05.T-04 Effects of Micropollutants and Their Degradation on Prokaryotic Communities at the Sediment–Water Interface

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Pesticides and pharmaceuticals frequently enter aquatic ecosystems as complex mixtures, complicating our understanding of their persistence, interactions, and ecological impacts. This study investigates the dissipation and effects of metformin (a widely prescribed antidiabetic drug), metolachlor (an agricultural herbicide), and terbutryn (a herbicide used in construction materials) on prokaryotic communities at the sediment water interface. Micropollutants were introduced either individually or as a mixture (17.6 µM per compound) using laboratory microcosms simulating the sediment water interface of aquatic systems. In a dedicated experiment, metformin degradation and its effect on prokaryotic communities were assessed under stable and variable oxygenation conditions in spiked laboratory microcosms. Metformin and metolachlor completely dissipated within 70 days, while terbutryn persisted, with no significant differences between single-compound and mixture exposures. Metformin showed a lag phase before undergoing rapid biotic degradation, yielding guanidylurea and dimethylamine as transient transformation

products, which may act as microbial carbon sources. The hypervariable V3?V4 region of the 16S rRNA gene was PCR amplified targeting both bacteria and archaea to evaluate the response of prokaryotic communities to micropollutant exposure. Prokaryotic community composition was strongly affected by the sediment and water matrix composition and incubation duration, with additional distinct effects induced by micropollutants. Recalcitrant compounds such as terbutryn and metolachlor in the mixture caused notable shifts in community structure. Interactions between oxygen conditions and metformin exposure were primarily additive, though some taxa exhibited synergistic or antagonistic responses, enabling the identification of potential microbial biomarkers of contamination. These findings underscore the intricate relationships between environmental conditions, micropollutant dynamics, and microbial ecology. The study highlights the need to account for non-additive interactions, microbial adaptations, and environmental factors, such as oxygen availability, when evaluating micropollutant degradation and ecological effects. By providing insights into micropollutant persistence and microbial community responses at the sediment-water interface, this research contributes to the understanding of ecosystem health and water quality management. This research and the fellowship of Adrien Borreca were funded by the CNRS 80|Prime program (2020-2023) and by the EU within the European Regional Development Fund (ERDF), support measure INTERREG VI in the Upper Rhine as part of the Reactive City A3-4 project (Towards a Reactive City without Biocides).

1.05.P-Tu027 Potential Adverse Effects of Plant Protection Products on Non-Target Organisms' Microbiome: Regulatory Challenges and Progress in the Environmental Risk Assessment

Franco Ferilli¹, Fernando Alvarez², Csaba Szentes² and Diana Di Gioia³, (1)European Food Safety Authority, Parma, Italy, (2)European Food Safety Authority, Italy, (3)University of Bologna, Italy

The potential adverse effects of plant protection products on non-target organisms microbiome has become an emerging concern due to the critical role microbiome has in regulating and supporting host health and resilience. Effects on the microbiome are not explicitly addressed under current environmental risk assessment frameworks. Nevertheless, in a recent assessment, the European Food Safety Authority scrutinised the open peer-reviewed literature on the effects of active substances and plant protection products on microbiome in the context of the peer review of glyphosate, evaluating the reliability and relevance of the identified studies. Studies highlighted different impacts on the gut, skin, and soil microbiomes. The available studies followed on non-standardized methods, hampering comparability and repeatability, and lacked direct applicability to regulatory risk assessments. A critical challenge was identifying core microbiomes, i.e., essential microbial communities underpinning an organism's health, and determining how shifts in these communities signal stress or harm. Variability in core microbiome composition across regions, seasons, and environmental conditions further complicates assessments. Moreover, linking microbiome disruptions at the individual level to broader population and ecosystem-level outcomes remains a significant challenge. EFSA's work underscores the need for standardized testing protocols, microbiome databases for non-target organisms, and methodologies to link microbiome-level to population-relevant effects. This work highlights both the challenges and opportunities for advancing the environmental risk assessment for non-target organisms.

1.05.P-Tu039 Do Microplastics Change the Microbial Risk Profile of Wastewater?

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Plastic pollution is pervasive and ubiquitous, and the harm it can cause is diverse. Impacts are dependent not only on polymer type or the associated chemicals (additives and non-intentionally added substance) but also size (nano- to macroplastics), morphotype, degree of aging and the organism and ecosystems with which they interact. Whilst there is an extensive body of knowledge of the physical and toxicological effects of plastics little is known of the effects on microbial function, or how two pollutants that pose a risk to human health - plastics and microbial pathogens interact, potentially altering the risk profile of both.

Wastewater treatment facilities act as a nexus for microbial pathogens and plastics. Depending on the level of treatment, plastic particles are removed from wastewater before discharge to the environment. Remaining particles are either released in treated effluent or enter the biosolids. As these plastics age they may leach and sorb pollutants and provide substrate for biofilm formation and optimal environment for conjugation, possibly impacting antimicrobial resistance (AMR) traits. To address the knowledge gap that exists for these plastic-microbe interactions we deployed five different artificially weathered plastic types and a glass control into the final maturation pond of a municipal wastewater treatment plant in Ōtautahi-Christchurch, Aotearoa New Zealand. The plastic-associated biofilms (plastisphere) were sampled at 2, 6, 26, and 52 weeks, along with the ambient pond water, at three different depths (20, 40, and 60 cm from

the pond water surface). Plastisphere microbial diversity and functional potential were determined using metagenomic sequence analysis.

Bacterial 16S ribosomal RNA genes composition did not vary among plastic types and glass controls but varied among sampling times. Overall, there was no polymer-substrate specificity evident in the total composition of genes, but sampling time and depth were significant factors. The plastisphere housed diverse AMR gene families, potentially influenced by biofilm-mediated conjugation. The health risk of plastic-associated microbes in the effluent may therefore pose an increasing environmental, and human health risk and warrants further study. This study highlights that the removal of microplastics from wastewater (ideally preventi) is critical for the protection of the environment and humans from both chemical and microbial threats. This study was supported by the New Zealand Ministry of Business, Innovation and Employment, Endeavour Fund Research Programme C03X1802 (Impacts of microplastics on New Zealand s bioheritage systems, environment, and eco-services).

1.05.P-Tu045 Assessing the Effects of Pollutants in Stormwater Wetlands on Sedimentary Microbial Community Structure Using eDNA Metabarcoding

Anna Flynn¹, Sara M Long¹, Vincent J. Pettigrove², Kathryn Hassell¹, Jeff Shimeta¹ and Mark Osborn¹, (1)RMIT University, Australia, (2)Aquest Research Group, RMIT University, Australia

Pollution poses a significant threat to freshwater ecosystems with metals and pesticides in particular representing long-term and widespread hazards. Constructed wetlands are an important tool for managing stormwater in urban landscapes, capturing and retaining contaminants in their sediments. Currently there is a lack of consideration for microbial taxa when determining the impacts of contamination on aquatic environments, and conversely an absence of microbial indices which could enhance current biomonitoring by increasing the scope and sensitivity of analyses. Environmental DNA is expanding the field of aquatic monitoring with the ability to assess biological profiles quickly and reliably, providing knowledge of how contamination affects ecosystems at a microbial level. By understanding what contaminants are in our waterways and how they affect all aspects of ecosystem functioning, we can better target mitigations to manage responses. In this study we collected sediment samples from the inlet and outlet of nine urban stormwater wetlands and one rural wetland, analysing them for chemical contaminants, and microbial community structure through 16S rRNA metabarcoding. Multivariate analyses were used to determine the interrelation between the chemical and biological data. We found significant variation in microbial communities between the rural wetland and all stormwater wetlands, between some of the stormwater wetlands, and between over half of the inlets to outlets. Cyanobacteria and Proteobacteria were mostly driving this variation, along with Planctomycetota and Bacteriodota. Seven families were also identified as having bioindicative potential for pollution in freshwater. We observed significant correlations between community structure and zinc and barium, with the latter not previously reported to be associated with microbial dynamics in freshwater. Our study further validates the use of eDNA metabarcoding to reliably evaluate sedimentary microbial profiles in freshwater, highlighting its value in the assessment and prediction of contamination in these environments. We identified pollutants of concern being seen commonly across urban environments in Melbourne and showed evidence that they are impacting the abundance and diversity of sedimentary microbial communities in stormwater wetlands. Through this we were able to identify potential microbial bioindicators which could be used in a biotic index as a measure of contamination in freshwaters.

1.05.P Microbiota Under Stress: Impacts of Environmental Pollutants on Ecosystem and Host Health

1.05.P-Tu027 Potential Adverse Effects of Plant protection products on Non-Target Organisms' Microbiome: Regulatory Challenges and Progress in the Environmental Risk Assessment

Franco Ferilli¹, Fernando Alvarez¹, Csaba Szentes¹ and Diana Di Gioia², (1)European Food Safety Authority, Italy, (2) University of Bologna, Italy

The potential adverse effects of plant protection products on non-target organisms microbiome has become an emerging concern due to the critical role microbiome has in regulating and? supporting host health and resilience. Effects on the microbiome are not explicitly addressed under current environmental risk assessment frameworks. Nevertheless, in a recent assessment, the European Food Safety Authority scrutinised the open peer-reviewed literature on the effects of active substances and plant protection products on microbiome in the context of the peer review of glyphosate, evaluating the reliability and relevance of the identified studies. Studies highlighted different impacts on the gut, skin, and soil microbiomes. The available studies followed on non-standardized methods, hampering comparability and repeatability, and lacked direct applicability to regulatory risk assessments. A critical challenge was identifying core microbiomes, i.e., essential microbial communities underpinning an organism s health,

and determining how shifts in these communities signal stress or harm. Variability in core microbiome composition across regions, seasons, and environmental conditions further complicates assessments. Moreover, linking microbiome disruptions at the individual level to broader population and ecosystem-level outcomes remains a significant challenge. EFSA's work underscores the need for standardized testing protocols, microbiome databases for non-target organisms, and methodologies to link microbiome-level to population-relevant effects. This work highlights both the challenges and opportunities for advancing the environmental risk assessment for non-target organisms.

1.05.P-Tu028 Impact of Engineered Nanomaterials on Microbial Communities in the Aquatic Environment

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Engineered nanoparticles have garnered significant attention for their unique properties and potential applications across various industries. They were extensively examined for their toxicological effect in vitro and in vivo, but their effects on environmental and biological systems remain underexplored. A key area of concern is how nanoparticles interact with microbial communities in natural ecosystems, since these communities are vital for processes such as nutrient cycling, biodegradation, and maintaining ecosystem health and functioning. Currently, environmental risk assessments of nanoparticles majorly involve higher organisms (e.g. algae, zooplankton), but omit the effects on complex communities and ecosystems as a whole. This research aims to investigate the impact of nanomaterials on aquatic microbial communities using a combination of laboratory experiments and field-sampling along a gradient from low to highly anthropogenically impacted aquatic ecosystems around Berlin, Germany. To holistically assess changes in microbial composition and functional activity in response to nanomaterial exposure, we will apply molecular, microbiological, analytical as well as OMIC methods. We hypothesize that nanoparticles induce significant compositional changes in microbial community structures, potentially leading to alterations in stress responses and other functional effects. Nanomaterials have the potential to benefit the environment by contributing to energy and resource efficiency, remediation of contaminated sites, or water treatment. In order to represent truly sustainable products, though, a safe use for humans and ecosystems must be assured. Ultimately, this research will contribute to more accurate environmental risk assessments and help guide the responsible use of nanotechnology in various industries.

1.05.P-Tu029 Combining Tools for Early Warning in Water Quality Monitoring

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In the Netherlands, water quality in the river is measured and monitored with a view to public health and state of the river-bound ecosystems. The Water Framework Directive (WFD) states that all the water in the Netherlands must be a good habitat for the organisms that live there by the year 2027 and that it must be reasonably easy to make drinking water from it.

However, measuring water quality is still in its infancy when it comes to the speed of the measurement, the reliability with regard to the predictive value and the still limited chemical and biological insight into the water composition. In this poster an innovative combination of existing tools that will be further developed will be shown. The combination of BACTcontrol and UV/VIS chemical fingerprint in a flowthrough system that continuously monitors the water quality will activate an alarm when a deviation in microbiological and/or chemical parameters is detected. This alarm also triggers an additional sample to be taken for Next Generation Sequencing (NGS) on DNA and RNA in the surface water in order to determine the biodiversity of the sample. All data from these analyses are combined with data like temperature, river discharge, nutrients, oxygen levels. All of the results are visible on a dashboard. This enables water quality managers to act in a targeted and preventive manner in the event of water pollution.

1.05.P-Tu030 Better or Worse? Assessing the Effects of Warming on Interactions Between Emerging Contaminants and Freshwater Primary Producers

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Freshwater is a finite resource for the human population and global economy that is increasing in demand. The services freshwater ecosystems supply for society is recognised through its value of \$4?trillion annually. However, these ecosystems face significant threats from various anthropogenic stressors released from point and non-point pollution sources that often act in combination rather than individually. While research into the effects of emerging contaminants has gained momentum, the impact of rising water temperatures due to climate change, alongside more frequent heatwaves, remains insufficiently explored. This study presents an initial systematic literature review aimed at evaluating existing research on the combined stressors of warming and emerging contaminants. The review revealed the variability in

synergistic or antagonistic effects depending on the biomarkers employed. Subsequently, key emerging contaminants (pharmaceuticals, pesticides, and heavy metals) were selected due to their risk assessed toxicity for further investigation through a laboratory study. Our experimental design involved assessing the interactions between these contaminants and freshwater primary producers, including phytoplankton, diatoms, and cyanobacteria, under varying temperature conditions in accordance with OECD 201 guidelines. Toxicity assessments were performed using effective concentration values derived from dose-response curves at three distinct temperatures: 15°C, 20°C, and 25°C. Initially nutrient enrichment will be studied first across the temperature rise on the primary producers to assess if it has an interactive effect before chemical exposure. In alignment with the literature's emphasis on the need for holistic approaches, a novel morphological analysis method was also employed to examine individual cellular responses. Preliminary results indicate that elevated temperatures significantly enhance the inhibitory effects of copper sulfate on the growth of the diatom *Nitzschia palea*. Additionally, higher temperatures exacerbated differences in chloroplast size between copper sulfate-exposed cells and controls, underscoring temperature's critical role in modulating contaminant toxicity at both population and cellular levels. The laboratory study is ongoing, and further analyses will contribute to a more comprehensive understanding of these interactions in freshwater ecosystems.

1.05.P-Tu031 Development of a Multispecies Test Method for Evaluating Phytoplankton Sensitivity to Chemical Exposure

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Phytoplankton are frequently used as model organisms in ecotoxicology studies due to their ecological importance, chemical sensitivity, and ease of cultivation in laboratory environments. They are essential for assessing the effects of pollutants on aquatic ecosystems. The toxicity of chemicals can vary greatly depending on the algal species. The OECD 201 guideline recommends just five species: two chlorophytes, *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*; two cyanobacteria, *Anabaena flos-aquae* and *Synechococcus leopoliensis*; and a single diatom, *Navicula pelliculosa*. However, there is a lack of representation and ecotoxicological data for some groups, particularly diatoms. This study aimed to develop a multispecies test method for assessing phytoplankton sensitivity to chemical exposure by examining a range of species, including three chlorophytes, two cyanobacteria, and seven diatoms. In the method, test organisms are exposed to a toxicant for 3 d with an automated microplate reader employed to measure optical density. Calibration curves were generated to establish the relationship between cell numbers and optical density, facilitating the conversion of optical density readings to cell numbers allowing the calculation of average specific growth rates according to the OECD 201 guideline. Potassium dichromate was used as a reference substance to validate the testing procedures and four UV filters were used as study toxicants. Chlorophytes were found to be the most sensitive group to potassium dichromate (EC₅₀=3.86-5.08 mg/L), while cyanobacteria and diatom species demonstrated EC₅₀ values ranging from 12.21 mg/L to 102 mg/L. EC₅₀ values were similar to values reported in the literature. For the UV filters, the diatom, *Aulacoseira granulate* (a species not in the OECD guideline) was the most sensitive species, with EC₅₀ values being 0.027 mg/L, 0.006 mg/L, and 0.295 mg/L for homosalate, octocrylene, and benzophenone-3, respectively. Bemotrizinol was found to have no effects at its solubility limit. The results show that this growth-based method can be applied to various algal species and chemical agents, adhering to OECD guidelines for algal sensitivity testing, including standard and non-standard species. The findings contribute to understanding the ecological impacts of chemicals on phytoplankton, emphasizing the need for further research to elucidate the implications for aquatic ecosystem health.

1.05.P-Tu032 Optical Microrespirometry: A Comprehensive Tool for Profiling Toxic Responses in Plankton Communities

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Optical microrespirometry is a potentially valuable tool in environmental toxicology for evaluating community level responses to environmental toxicants. Oxygen is a key molecule in the physiology of many macro- and microorganisms, and community level fluctuations in oxygen may indicate metabolic perturbations in response to chemical stressors. This study explored the potential of an optical microplate respiration system for assessing metabolic responses of microplankton communities to toxicant exposure. The microrespirometry system was based on multi-well glass microplates equipped with oxygen sensor spots for non-invasive monitoring of oxygen levels in real time. Mixed freshwater plankton communities were obtained from oligotrophic, mesotrophic and eutrophic lakes in North Jutland (Denmark). Toxicants included pesticides and biocides such as glyphosate, chlorhexidine and benzalkonium chloride in different concentrations. Multivariate statistics was used to analyse data generated during microrespirometry profiling. The results showed differences in aerobic metabolism of native plankton communities that

corresponded to toxicant levels. In addition, functional groups of autotrophic and heterotrophic plankton organisms were activated by addition of low levels of inorganic or organic substrates and use of different light regimes (substrate induced respiration). It was subsequently possible to approximate the toxic responses of different functional groups in the plankton community. Changes in oxygen consumption of mixed communities often occurred in phases, and apparent community level EC50 values were calculated for both an initial respiration phase (hours) and a subsequent respiration phase (days). Microrespirometry based toxicity was evaluated further by measuring the efficacy of an advanced oxidation process in reducing apparent ecotoxicity. Water contaminated with biocides were treated with vacuum UV irradiation and responses in lake water was assessed before and after treatment. The results indicated that plankton microrespirometry could also be expanded to assess the environmental quality of water treated for reuse. Collectively, the study indicates that optical microrespirometry may be used as a sensitive and non-invasive proxy to assess community level inhibitory responses of lake plankton in real time. The methods enabled comprehensive profiling of plankton responses across toxicant concentrations, substrate conditions and trophic levels. This project has received funding from the European Union under the Horizon Europe Marie Skłodowska-Curie Actions (MSCA) Doctoral Network program (Project IN2AQUAS; Grant agreement number 101119555).

1.05.P-Tu033 How Sensitive are Aquatic Fungi? A Case Study in the Light of Environmental Risk Assessment Using the Fungicide Trifloxystrobin as Model

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Aquatic hyphomycetes are anamorphic fungi usually found associated with decomposing organic substrates such as leaves and twigs. This association suggests these fungi are crucial for nutrient cycling, decomposing complex organic matter and enhancing the feeding and nutrition of higher trophic levels (i.e., detritivores). Despite their key role in ecosystem functions, ecotoxicological assessments for this group are limited, surprisingly, even for fungicides, a group of pesticides designed to control fungal (pest) species. By employing the fungicide trifloxystrobin as a model, this study aims to assess the value of testing non-target fungal species, here aquatic hyphomycetes, for environmental risk assessment. This was done by developing a species sensitivity distribution (SSD) from growth inhibition effect concentrations (EC_x). We exposed eleven aquatic hyphomycete species, belonging to five different orders and grown on malt extract agar, to increasing concentrations of trifloxystrobin (up to 625 µg/L) in the darkness at 16 °C for three weeks. Growth in terms of radial growth was measured weekly. EC values increased with exposure duration. Overall, EC₁₀ values varied between 0.27 and 505 µg/L and EC₂₅ between 1.53 and 504 µg/L. In the case of EC₅₀ values, 6 of the 11 species tested had estimates above the tested concentration range (> 625 µg/L). Among the other five species, the EC₅₀ values varied between 2.48 and 408 µg/L. There was no fungal order that stood out in its general sensitivity towards trifloxystrobin. Moreover, the hazardous concentrations for 5% of the species (H₅), abstracted from the SSD curves with EC₁₀ values, were estimated at 7d = 0.21 µg/L, 14d = 0.17 µg/L and 21d = 0.09 µg/L. Considering the lowest EC₁₀ value and the maximum assessment factor (AF) for SSD curves, we calculated a Predicted No Effect Concentration (PNEC) of 0.05 µg/L for aquatic hyphomycetes, which is lower than the regulatory acceptable concentration (RAC) for trifloxystrobin in the European Union (0.11 µg/L), a scenario that would be enough to pose a risk to the most sensitive species. This reveals a gap between current regulations and the sensitivity of hyphomycetes to fungicides. To address this, further studies are needed, evaluating key substances as well as other endpoints. Such research could guide updates in environmental risk assessments, ensuring regulations fully encompass the diversity and ecological functions of freshwater habitats. CAPES-Humboldt Research Fellowship (grant n°. 88881.699331/2022-01)

1.05.P-Tu034 Spatiotemporal Dynamics of Riverine Benthic Microbial Communities and their Biodegradation Potential

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Pollution is a well-known cause of drastic ecosystem change, devastatingly affecting freshwater habitats. Despite recent progress in chemical and bioanalytical techniques, our understanding of the effects of chemical exposure on biological communities and ecosystems remains limited. The natural variability in species composition, ecological interactions, and complex mixtures of chemical pollutants impede the

development of a standardizable and systematic approach to monitoring and assessing the impacts of freshwater pollution in impacted habitats. Most research focuses on how biological communities respond to pollutants, e.g., wastewater discharge, by examining shifts in community composition and diversity within specific groups. Benthic communities, particularly microorganisms, are naturally subjected to spatial and temporal variability. However, additional investigation is needed to evaluate how wastewater effluents affect the spatiotemporal dynamics of biodiversity and their associated functions. Exploring spatial variations and seasonal community dynamics in affected habitats can enhance our understanding of the link between the biodiversity of key organisms and their role in ecosystem processes (e.g., contaminant biodegradation). Using environmental DNA analysis, we assess the spatiotemporal dynamics of benthic community diversity and composition, i.e., prokaryotic, algae, fungi, protists, and metazoans, and their association with biodegradation potential from two wastewater-impacted rivers in Sweden. We also quantified ecological processes, e.g., stochastic and deterministic, regulating the seasonal assembly of the benthic communities. We aimed to understand the spatial and seasonal organization and dynamics of multitrophic community assembly and assess their link to the biodegradation capacities of the microbial communities in wastewater-impacted rivers.

1.05.P-Tu035 Does Exposure History Affect the Sensitivity of Sediment-Associated Microorganisms?

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Inland waters are regarded as one of the most important production grounds of the greenhouse gas methane (CH₄). The decomposition of organic matter depletes most of the oxygen in the sediment, resulting in anaerobic conditions, promoting microbial methanogenesis. As CH₄ emissions from natural sources are increasing, examining the environmental factors that influence the increase, is of great relevance in the context of global change. A previous study has shown that the exposure of sediment-associated microorganisms to pharmaceuticals (i.e., an antibiotic mixture) stimulates the emissions of CH₄, by affecting the methanogenesis in freshwater sediment. Here, we assess whether the exposure history of sediment-associated microorganisms in freshwaters modifies their sensitivity to increasing levels of pharmaceuticals reflected by differences in CH₄ production. Differences in exposure history were assumed by sampling sediment-associated microorganisms at different sites, up- and downstream of wastewater treatment plants (WWTPs), which are point sources of pollution for a wide range of organic and inorganic chemicals. In detail, four streams in the upper Rhine valley were sampled at four sites in each stream: a pristine area, directly upstream of the WWTPs effluent, 50 m and 1 km downstream of the effluent. In the laboratory, sediment was exposed to an antibiotic mixture (i.e. amoxicillin, ciprofloxacin, erythromycin, sulfamethoxazole, and tetracycline) in a hermetically closed microcosm under anaerobic conditions. Two treatment levels, i.e. 5 and 5000 µg/L, in addition to the negative control are tested with 10 replicates per treatment. The systems are incubated at 20 ± 1 °C in the dark, and CH₄ and CO₂ in the headspace are currently measured on a weekly basis, until production asymptotically approaches the maximum levels. Metabarcoding will shed light on changes in the microbial communities throughout the study. Data collection and analyses are still ongoing.

1.05.P-Tu036 Interactive Effects of Pollutants and Parasites on Cyanobacterial Metabolism

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Water pollution from chemical substances, such as the herbicide metolachlor (MET), and solid waste, such as cigarette butts (CBs), poses significant risks to aquatic ecosystems. For instance, MET can disrupt the growth and general metabolism of non-target microorganisms. Similarly, CBs, one of the most common forms of global litter, contain various chemicals that leach into water, negatively affecting aquatic microorganisms. Despite their prevalence, little is known about the combined effects of these pollutants and biotic stressors, such as parasites, on phytoplankton. This study investigated the metabolic response of the toxigenic cyanobacterium *Planktothrix agardhii* exposed to both biotic (the chytrid parasite *Rhizophydium megarrhizum*) and abiotic stressors (CB leachate and MET). We measured key metabolic biomarkers, including microcystin levels, protein and lipid content, lipid peroxidation, and the enzymatic activity of superoxide dismutase (SOD) and glutathione-S-transferase (GST). MET exposure did not significantly alter protein or lipid content or enzyme activities in either infected or uninfected cultures. However, cyanobacteria exposed to both MET and chytrids exhibited reduced microcystin levels and lipoperoxidation, indicating an oxidative stress response countered by non-enzymatic mechanisms. In contrast, CB leachate reduced protein and lipid content and microcystin concentrations in the

cyanobacteria only in the absence of the parasite, suggesting oxidative stress and metabolic disruption. Interestingly, simultaneous exposure to CB leachate and chytrids appeared to mitigate the negative effects. This study highlights the complex responses of cyanobacteria under combined abiotic and biotic stressors, revealing how anthropogenic pollutants shape aquatic microbial interactions and broader ecological processes. This work was supported by the DFG grant MA 9934/1-1 (EMR)

1.05.P-Tu037 Toxicity of ‘Eco-friendly’ Plasticizers on Plastic-Degrading Bacteria: Effects on microbial biofilm formation and Oxidative Stress Response

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Plasticizers, widely used to enhance the flexibility and durability of polyvinyl chloride (PVC), have raised environmental and health concerns due to their potential toxicity. Traditional phthalates, like di(2-ethylhexyl) phthalate (DEHP), are notorious for their harmful effects, prompting the development of alternative plasticizers such as dioctyl terephthalate (DOTP) and acetyl tributyl citrate (ATBC), which are marketed as safer, eco-friendly options. However, the impact of these alternatives on microbial communities, especially those involved in plastic degradation, is not well understood. This study examines the effects of DOTP and ATBC on *Rhodococcus ruber*, a bacterium with plastic-degrading potential. Using live/dead staining and CellROX Green reagent, we assess bacterial viability and oxidative stress at various concentrations of DOTP (1%, 5%, and 10% v/v) and ATBC (1%, 5%, 10%, and 20% v/v), reflecting concentrations commonly found in plastic products (ranging from 1 to 20% v/v). Our findings reveal that both plasticizers cause significant harm to *R. ruber*, with increasing concentrations leading to higher mortality rates and oxidative stress. At 10% DOTP and 20% ATBC, no live cells were detected. These results highlight the need to better understand the environmental risks posed by alternative plasticizers and their potential impact on the plastisphere.

1.05.P-Tu038 Exploring the Biodegradation of Microplastics Using Gut Microbiota from Freshwater Insects

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Microplastics in aquatic ecosystems pose significant risks to biodiversity and ecosystem health. Addressing this challenge requires sustainable and innovative strategies, such as leveraging microorganisms associated with aquatic organisms for bioremediation. This study investigates the gut microbiota of *Chironomus riparius* larvae as a potential source of bacterial strains capable of degrading microplastics. Bacterial strains isolated from the larval gut were tested for their ability to grow on minimal media supplemented with polyethylene (PE), polyvinyl chloride (PVC), and polyamide (PA) as the sole carbon sources. The ability to utilize microplastics was assessed by monitoring bacterial growth intensity. Notably, strains of *Peribacillus simplex* and *P. frigoritolerans* exhibited high growth on all three types of microplastics. Furthermore, *Bacillus wiedmannii* and *B. thuringiensis/toyonensis* demonstrated moderate degradation activity, indicating a potential role in breaking down recalcitrant polymers. Some additional strains, such as *Paenibacillus xylanexedens*, displayed limited plastic degradation. These findings highlight the potential of *C. riparius* gut microbiota as a reservoir of microplastic-degrading bacteria and underscore the importance of exploring natural microbial communities for sustainable bioremediation solutions. Future work will focus on scaling up these processes for broader environmental applications.

1.05.P-Tu039 Do Microplastics Change the Microbial Risk Profile of Wastewater?

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Plastic pollution is pervasive and ubiquitous, and the harm it can cause is diverse. Impacts are dependent not only on polymer type or the associated chemicals (additives and non-intentionally added substance) but also size (nano- to macroplastics), morphotype, degree of aging and the organism and ecosystems with which they interact. Whilst there is an extensive body of knowledge of the physical and toxicological effects of plastics little is known of the effects on microbial function, or how two pollutants that pose a risk to human health - plastics and microbial pathogens interact, potentially altering the risk profile of both.

Wastewater treatment facilities act as a nexus for microbial pathogens and plastics. Depending on the level of treatment, plastic particles are removed from wastewater before discharge to the environment. Remaining particles are either released in treated effluent or enter the biosolids. As these plastics age they may leach and sorb pollutants and provide substrate for biofilm formation and optimal environment for

conjugation, possibly impacting antimicrobial resistance (AMR) traits. To address the knowledge gap that exists for these plastic-microbe interactions we deployed five different artificially weathered plastic types and a glass control into the final maturation pond of a municipal wastewater treatment plant in Taupō, New Zealand. The plastic-associated biofilms (plastisphere) were sampled at 2, 6, 26, and 52 weeks, along with the ambient pond water, at three different depths (20, 40, and 60 cm from the pond water surface). Plastisphere microbial diversity and functional potential were determined using metagenomic sequence analysis.

Bacterial 16S ribosomal RNA genes composition did not vary among plastic types and glass controls but varied among sampling times. Overall, there was no polymer-substrate specificity evident in the total composition of genes, but sampling time and depth were significant factors. The plastisphere housed diverse AMR gene families, potentially influenced by biofilm-mediated conjugation. The health risk of plastic-associated microbes in the effluent may therefore pose an increasing environmental, and human health risk and warrants further study. This study highlights that the removal of microplastics from wastewater (ideally prevented) is critical for the protection of the environment and humans from both chemical and microbial threats. This study was supported by the New Zealand Ministry of Business, Innovation and Employment, Endeavour Fund Research Programme C03X1802 (Impacts of microplastics on New Zealand's bioheritage systems, environment, and eco-services).

1.05.P-Tu040 Microbial Degradation Potential of Hydrocarbon Contaminants in the Baltic Sea
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The Baltic Sea is loaded with petroleum-based substances, e.g., hydrocarbons and plastic waste, from various sources. Microbial communities drive organic matter degradation and can naturally degrade environmental pollutants. The information on the degradation potential of contaminant-exposed microbial communities can be used to estimate persistence or to predict the fate or accumulation of petroleum-based pollutants in the environment. This would be useful in managing and mitigating the crisis in crude oil and plastic pollution in the Baltic region. However, there is still limited information on the degradation potential of microbial communities in the Baltic Sea, alongside the lack of environmental monitoring information on these contaminants. This prompts the need for a comprehensive profile of the region's microbial degradation capacity. In this study, we compiled metagenomic datasets, profiled the microbial community structure, and identified hydrocarbon degradation genes from benthic sediment and water column samples collected from the Baltic Sea. We report the taxonomic and functional information on the potential of Baltic Sea microbes to degrade various types of hydrocarbon contaminants. Notably, metagenome-assembled genome taxonomy revealed that known hydrocarbon-degrading taxa (e.g., UBA9160 and Acidimicrobiales) were present in both benthic and pelagic environments of the Baltic. Aerobic degradation was also the most prominent and abundant degradation pathway in all environmental samples. The microbial taxa associated with hydrocarbon degradation showed variations in their substrate preferences between environmental samples and across the Baltic Sea basin. Long-chain alkanes and dibenzothiophene are the preferred substrates of hydrocarbon degraders. Our report aims to deepen our understanding of microbial degradation of contaminants that would be useful for improving mitigation strategies for crude oil spills and plastic pollution in the Baltic Sea while addressing critical environmental concerns.

1.05.P-Tu041 Sea urchin Microbiota/Immune Cell Model for Assessing the Mutual Immune Response to Nanoparticles

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Each nanomaterial exhibits novel, cutting-edge features whose benefits depend on their safety and biocompatibility. The innate immune system acts as the first player involved in the recognition/interaction of nanomaterials, representing the main hub for explaining the safety of each new nanomaterial. Still, it is not the only system involved. The co-evolution of the microbiota with the innate immune system built an interdependence regulating immune homeostasis that is poorly studied, mainly because of the practical

and ethical difficulties of studies in mammalian models. Herein, we propose a new investigative strategy for the assessment of potential toxicity/safety concerns regarding Iron-Oxide Nanoparticles (Fe-oxide NPs) in the Fe₂O₃ structure, known to be used in several biomedical and environmental applications, by investigating the simultaneous interaction of particles, immune cells, and the microbiota associated with the blood of the sea urchin in a natural co-culture system, where cell-cell signaling remains preserved. Sea urchins share with human beings many gene families and biological pathways involved in innate immune defense but also a complex microbial community with a high taxonomic composition, including bacteria colonizing the human gut. The simultaneous interaction of particles, immune cells, and the associated microbiota is addressed in vitro-ex vivo by applying a wide-ranging approach, including Raman spectroscopy, 16S Next-Generation Sequencing, Cell Painting, and NanoString nCounter. Our findings highlight a shift in the composition of microbial groups associated with the molecular reprogramming of the sea urchin immune cells exposed to Fe-oxide NPs and an increase in extracellular vesicles released in the culture medium upon insult. A tolerogenic multi-level immune cell-microbiota response is designed to restore homeostasis and maintain the system in physiological balance by immune cell/microbiota conditioning (mutual immune response). Based on the evidence of the similarities between sea urchins and humans, the sea urchin microbiota/immune cell model becomes an excellent interactive proxy-to-human system for probing the immunological safety of NPs in environmentally relevant conditions and limiting animal use. This work has been financed by the European Union (Next-generation EU), PNRR, M.4-C2-1.1 through the MUR-PNRR PRIN 2022 project SURPRISE (GA P2022LASKT) and the MUR-PNRR project SAMOTHRACE (GA ECS00000022).

1.05.P-Tu042 Impact of Mining-Derived Metal Contamination on Fish Parasites at Tar Creek Superfund Site, Oklahoma

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The Tri-State Tar Creek Mining District, once a lead and zinc mining hub, now suffers from severe contamination, with metals like lead, cadmium, and zinc leaking into the soil and water. This study examines how metal pollution influences fish parasitic loads and the relationship between fish and their parasites in these stressed environments. Parasites are more sensitive to metals than fish, but specific adaptive mechanisms are poorly understood. Moreover, little is known about how metal exposure affects parasite diversity and health. This research enhances our understanding of metal pollution's impact on parasite populations, communities, and fish-host interactions. Field sampling took place at four sites across the Grand Lake area in Oklahoma: two reference sites (Grand Lake Honey Creek & Sycamore Creek) and two polluted sites (Tar Creek E40 and Miami, OK). Water analysis showed a clear gradient of metal contamination, with zinc levels ranging from 1.1 µg/L at reference sites to 7,000 µg/L at polluted sites. Cadmium ranged from 0.10 µg/L at reference sites to over 12 µg/L at polluted locations, and lead levels were 1.8 µg/L at reference sites and 2.5 µg/L at polluted sites. Fish were euthanized, dissected for parasites, and species richness and parasitic load was recorded. The most common parasite, the metacercariae stage of the trematode *Posthodiplostomum* sp., was isolated for metal analysis. A pool of fifty to seventy-five metacercariae was dried and acidified for inductively coupled plasma mass spectrometry analysis (ICP-MS). Preliminary results suggest fish persist even in highly contaminated sites, but parasite species richness is notably lower at polluted sites (Miami/E40: $2.2 \pm 1.1/1.0 \pm 0.7$) compared to reference sites (GLHC/SYC: $2.4 \pm 0.9/2.4 \pm 0.547$). Parasitic load differed significantly, with fish from polluted sites hosting distinct parasite communities and an average of 109 parasites per fish, compared to 252 parasites per fish in fish from cleaner sites.

The study aims to determine if water contamination levels correlate with parasite metal bioaccumulation and diversity changes. It explores how parasitic load differs between clean and polluted sites, whether parasites accumulate metals differently from fish, and if fish and parasites co-evolve to tolerate metal exposure. By examining these relationships with water chemistry, the research addresses fundamental questions of cross-species adaptation to metal pollution.

1.05.P-Tu043 Ecotoxicological Assessment of a copper-based Nanopesticide toward *Xenopus laevis* and its Gut Microbiota

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Copper nanoparticles are widely used for their antibacterial and antifungal properties, especially in

nanopesticides for agricultural applications. Their release from soil to aquatic environments might threaten non-target organisms. Amphibians have already been heavily impacted by anthropogenic activities and their associated gut microbiota are likely to be sensitive to products such as copper nanoparticles. Ecotoxicological data and studies on commercial formulations that specifically focused on aquatic systems are still scarce. The aim of this study was to determine the impact of a copper ($\text{Cu}(\text{OH})_2$)-based nanopesticide, Kocide 3000® (K3), in comparison to Kocide 2000® (K2), a non-nanosized equivalent, at environmentally relevant concentrations (0.005, 0.010, 0.025 and 0.050 mg Cu/L). For this purpose, *X. laevis* tadpoles were exposed for 12 days, after which several endpoints were measured, and 16S rDNA gene sequencing was performed to characterize the bacterial composition in the gut. Copper salt ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) was also tested to provide a benchmark for assessing copper-related toxicity alongside the complex commercial formulations. Both Kocide® products were found to inhibit the growth of exposed organisms with a significant effect at 0.050 mg Cu/L. Analyses showed that all contaminants, regardless of the concentration, induced erythrocyte cell cycle disturbances. The results obtained with the micronucleus assay demonstrated the genotoxicity of the nanopesticide K3. Regarding the intestinal microbiota response, no effect on alpha diversity was observed at the tested doses. However, bacterial communities were shown to be significantly altered. The effect of the two commercial products differs from that of copper alone, inducing a differential modification of the bacterial community structure, visible in the abundance of phyla. Only the nanopesticide K3 induced significant changes compared to the negative control at all tested concentrations. This study highlights the effects of copper nanopesticides on aquatic organisms and their associated microbiota, contributing to the understanding of microbial responses to environmental stressors. It also emphasizes the need for further research into the mechanisms underlying the genotoxic and microbiota-related impacts of copper nanopesticides, as well as their broader ecological consequences following agricultural use.

1.05.P-Tu044 Effects of Graphene Oxide on *Xenopus Laevis* After Multi Pathway Exposure: Focus on Host Associated Microbiomes

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Graphene-based nanomaterials, such as graphene oxide (GO), possess unique properties that have spurred their application in industrial and medical fields. However, GO is likely to be released into aquatic ecosystems during its life cycle, necessitating a thorough evaluation of its ecotoxicological risks to ensure its safe use. Amphibians are particularly sensitive to environmental contaminants, and previous studies have demonstrated various toxic effects of GO, including genotoxicity, primarily in larval stages through direct exposure. This study extends the scope by investigating the combined effects of direct and trophic GO exposure on post-metamorphic *Xenopus laevis*, with a focus on its impact on microbiomes critical for host homeostasis, nutrient absorption, and immunity. To simulate realistic exposure scenarios, dipteran larvae were exposed to different concentrations of GO for 24 hours before being introduced as prey to juvenile *Xenopus*. These amphibians ingested the GO-laden larvae while also being exposed to GO in the water column. Genomic DNA was extracted from the entire intestine and a skin fragment of *Xenopus* after 24 and 96 hours to analyze bacterial community composition. The microbiota of the dipteran larvae was also characterized. The results revealed GO-induced shifts in the abundance of key intestinal phyla, such as Firmicutes and Bacteroidetes, which are often associated with dysbiosis. On the skin, significant alterations in microbiome composition were observed, including changes in Proteobacteria, a phylum crucial for resistance to pathogens like *Batrachochytrium dendrobatidis*. Additionally, the microbiota of dipteran larvae, representing the lower trophic level, was affected by GO exposure. This study highlights the potential ecological consequences of GO contamination at environmentally relevant concentrations, demonstrating its disruptive effects on microbiomes under realistic exposure conditions. The findings underscore the importance of integrating microbiome analyses into ecotoxicological studies to account for indirect effects that may have significant ecological implications.

1.05.P-Tu045 Assessing the Effects of Pollutants in Stormwater Wetlands on Sedimentary Microbial Community Structure Using eDNA Metabarcoding

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Pollution poses a significant threat to freshwater ecosystems with metals and pesticides in particular representing long-term and widespread hazards. Constructed wetlands are an important tool for managing stormwater in urban landscapes, capturing and retaining contaminants in their sediments. Currently there is a lack of consideration for microbial taxa when determining the impacts of contamination on aquatic

environments, and conversely an absence of microbial indices which could enhance current biomonitoring by increasing the scope and sensitivity of analyses. Environmental DNA is expanding the field of aquatic monitoring with the ability to assess biological profiles quickly and reliably, providing knowledge of how contamination affects ecosystems at a microbial level. By understanding what contaminants are in our waterways and how they affect all aspects of ecosystem functioning, we can better target mitigations to manage responses. In this study we collected sediment samples from the inlet and outlet of nine urban stormwater wetlands and one rural wetland, analysing them for chemical contaminants, and microbial community structure through 16S rRNA metabarcoding. Multivariate analyses were used to determine the interrelation between the chemical and biological data. We found significant variation in microbial communities between the rural wetland and all stormwater wetlands, between some of the stormwater wetlands, and between over half of the inlets to outlets. Cyanobacteria and Proteobacteria were mostly driving this variation, along with Planctomycetota and Bacteroidota. Seven families were also identified as having bioindicative potential for pollution in freshwater. We observed significant correlations between community structure and zinc and barium, with the latter not previously reported to be associated with microbial dynamics in freshwater. Our study further validates the use of eDNA metabarcoding to reliably evaluate sedimentary microbial profiles in freshwater, highlighting its value in the assessment and prediction of contamination in these environments. We identified pollutants of concern being seen commonly across urban environments in Melbourne and showed evidence that they are impacting the abundance and diversity of sedimentary microbial communities in stormwater wetlands. Through this we were able to identify potential microbial bioindicators which could be used in a biotic index as a measure of contamination in freshwaters.

1.05.P-Tu046 Soil Microbial Ecotoxicology – Protecting Ecosystem Key Players and Preserving Ecosystem Services

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This presentation will explore current requirements under various existing EU regulations to assess ecotoxicological impact on microbial communities. It will delve into the regulations and compare with the latest scientific studies and research to identify whether microbial communities are sufficiently protected from hazards of chemicals emitted to the environment. Any gaps that are identified will be discussed with regard to the effects that chemical pollutants may have on soil microbial communities. We will explore subsequent ripple effects that extend from microbial communities to other organisms within the ecosystem and ultimately on the functionality of the ecosystem itself. Microbial communities are the basis of most ecosystems on Earth, ranging from deep-sea vents over planktonic communities to various soil ecosystems. Colonization by microorganisms and their metabolic impact make these ecosystems available to other organisms and in many cases are a driving force behind the diverse ecosystems that are found around the world. As a result, disturbance to those sometimes very sensitive microbial communities, can have far reaching effects, impacting biodiversity, ecosystem function and finally ecosystem services. It is known that microorganisms can react radically to a changed chemical environment by using different nutrients or changing their metabolism based on availability of different electron acceptors/donors. While this is well known for lab cultures and has been extensively studied in the lab for numerous microbial communities, the far-reaching impacts this might have on a global scale are poorly understood. Chemical pollution may for example, lead to toxicity effects, changing the composition of microbial communities or impact nutrient availability. With molecular tools becoming increasingly available over the past two decades, we now have the tools to study microbial ecotoxicology in the field using a combination of various incubation methods combined with molecular and sequencing approaches. In this presentation I will provide an outlook on how experimental methods may be used to understand the impact of chemical pollution on microbial communities in selected environments. Comparing this with the initial review of the current regulatory status, we will understand which gaps will need to be addressed from a regulatory perspective to protect microbial communities in the environment and preserve their critical function within ecosystems.

1.05.P-Tu047 High-Throughput Screening of Organic Contaminants Affecting Host-Associated and Environmental Microbiomes

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Tire chemicals and per- and polyfluoroalkyl substances (PFAS) are among the most concerning

environmental pollutants due to their persistence, widespread distribution, and harmful effects. While traditional (eco)toxicological studies often focus on in vitro and in vivo models, they typically involve a limited selection of organisms, tissues or cells, with little attention is given to microbial communities. However, microbiomes are crucial for maintaining human health and ecological balance, playing vital roles in immune function, nutrient cycling and pathogen resistance in both human and environmental systems. Exposure to xenobiotics, such as persistent or toxic pollutants, can disrupt microbiome composition and is a potential driver of shifts in microbial diversity and function. To address this, we conducted a comprehensive screening of the effects of tire chemicals and PFAS on both host-associated (human gut and lung) and free-living (soil) microbiomes. To systematically investigate chemical contaminant-microbiome interactions across diverse systems, we evaluated the growth of about 60 representative bacterial, archaeal, and fungal strains sourced from human gut, lung, and soil habitats, following exposure to 30 chemicals (tire additives and PFAS) and their mixtures. Our overarching goal is to provide the first comprehensive, cross-system understanding of the impact of emerging pollutants on human and environmental health by elucidating how microbial growth is affected by exposure to tire chemicals and PFAS across diverse habitats. Authors acknowledge funding from the Austrian Science Fund, Cluster of Excellence CoE7, Grant-DOI 10.55776/COE7.

1.06.A Data-Driven Toxicology: Practical Applications and Insights into Using Existing and Emerging Data in Support of Human and Environmental Health

1.06.A.T-01 FAIR Toxicology Data Approaches Being Developed in PARC

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Risk Assessment, whether for humans or the environment, requires access to high quality data regarding both exposure and hazard of chemicals, which presents a significant challenge currently as each sub-domain (exposure, toxicology, human and environmental (bio)monitoring) has its own methodological approaches and conventions in how to collect, capture, store and share data, and these are not necessarily compatible (interoperable in technical terms). A major effort within PARC is devoted to developing and optimising the working practices, and technical solutions, to enable integration of disparate data for enhanced chemical risk assessment and responsive regulation. Toxicology data spans a huge range of approaches (in vitro, in vivo, in silico), end-points (apical / regulatory, mechanistic), timescales (acute, chronic, repeat dose, post-exposure monitoring, surveillance), data types (omics, image, summary etc.) and organisational frameworks (e.g., adverse outcome pathways). Here we present the PARC Toxicology cluster activities, including landscape mapping existing approaches (inventory of FAIR Enabling Resources for Toxicology), the PARC metadata at investigation, study and assay (ISA-Tab) levels and metadata capture templates and code-book, and the development of new metadata capture templates for end-points not yet covered in the OECD Harmonized Templates. Within the PARC Toxicology cluster activities to make toxicology data FAIR (Findable, Accessible, Interoperable and Re-usable) are grouped under 4 key areas - regulatory toxicity, omics, images and AOP. While each have unique challenges and objectives, there are a lot of commonalities across the approaches and tools available to support FAIRification of these data, such that the clustering will ensure cross-talk and sharing of best practices across areas. A toxicology-wide landscape mapping has been performed to capture the full spectrum of FAIR Enabling and FAIR Supporting resources, which has been published as the PARC Toxicology FAIR Implementation profile, and will be presented along with a FAIR Convergene Matrix showing commonalities with other projects / efforts working on toxicology, including the ASPIS project cluster, and gaps where no FAIR Enabling Resources exist, as a means to prioritise our FAIR-Tooling development efforts. This is a living document that will be continuously updated as new tools or approaches emerge, or as tools or approaches become obsolete in this fast-moving field. Funded via the Horizon-Europe Partnership for Assessment of the Risks of Chemicals (PARC, Grant Agreement No. 101057014), with co-funding for UK contribution via Innovate UK (project ID 1003384).

1.06.A.T-02 Precision Environmental Health: A Data-Driven Approach That Identifies Hazards of Complex Chemical Mixtures in the Environment

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The assessment and regulation of chemical toxicity to protect human health and the environment is done one chemical at a time and seldom at environmentally relevant concentrations. However, chemicals are found in the environment as mixtures, and their toxicity is largely unknown. Understanding the hazard posed by chemicals within the mixture is critical to enforce protective measures.

Here, we demonstrate the application of a data-driven, hypothesis-free approach to the sentinel and

ecotoxicology model species *Daphnia* to reveal the biomolecular response induced by real-world mixtures. We exposed a *Daphnia* strain to the river waters of the Chaobai River and measured gene expression profiles. Using a multi-block correlation analysis, we establish correlations between chemical mixtures identified in 30 water samples with gene expression patterns induced by these chemical mixtures. We identified 80 metabolic pathways putatively activated by mixtures of inorganic ions, heavy metals, polycyclic aromatic hydrocarbons, industrial chemicals, and a set of biocides, pesticides and pharmacologically active substances. Our data-driven approach discovered both known bioactivity signatures with previously described modes of action and new pathways linked to undiscovered potential hazards. This study demonstrates the feasibility of reducing the complexity of real-world mixture toxicity to characterise the biomolecular effects of defined chemical components based on gene expression monitoring of the sentinel species *Daphnia*. This work was supported by the Royal Society International Collaboration Award (grant No IC160121), the European Union's Horizon 2020 research and innovation programme (grant No. 965406), the National Natural Science Foundation of China (grant No. U20A20133), and the Natural Environmental Research Council Innovation People (grant No. NE/Y005120/1).

1.06.A.T-03 Ecotoxicity Prediction of Plastic Additives and Their Alternatives Using ToxCast/Tox21 Data-Based Machine Learning Models Within a Cross-Species Adverse Outcome Pathway Framework

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Plastic pollution is a critical global issue, with micro- and nano-sized particles from degrading plastics leaching additive chemicals into the environment, contributing significantly to toxicity. While hazards of legacy plastic additives like DEHP are known, ecotoxicity data for many alternatives remain limited. Large repositories of in vitro high-throughput screening data, such as the US EPA's ToxCast, offer valuable resources, but their application has largely been limited to human risk assessment rather than ecotoxicological contexts. The adverse outcome pathway (AOP) framework offers a promising approach to bridging this gap by linking molecular toxicity endpoints to apical effects in both humans and ecological species. This study aimed to identify cross-species AOPs relevant to plastic additives and develop machine learning models for predicting the ecotoxicity of alternative plasticizers. The in vitro response of plastic additives in human cells was examined, and conserved mechanisms were identified using SeqAPASS. These mechanisms were compared to in vivo ecotoxicity data from the ECOTOX database to determine their relevance to apical toxicity in ecological species. Based on this analysis, potential cross-species AOPs were identified. Machine learning models were then trained using molecular fingerprints and datasets integrating ToxCast bioassays with ECOTOX data. Model performance was assessed through five-fold cross-validation and in-loop validation. Mechanisms related to androgenicity leading to developmental and reproductive toxicity were conserved across humans and fish. For chemicals tested in both in vitro ToxCast/Tox21 assays and in vivo studies, bioactive concentrations (AC50) for androgen receptor binding and transcription were significantly correlated with the lowest effect levels (LELs) observed in ecological species. These findings informed the development of cross-species AOPs. Machine learning models trained on in vitro and in vivo datasets achieved high performance, with AUC-ROC values ranging from 0.68 to 0.92. This study demonstrated the utility of integrating ToxCast/Tox21 bioassays and AOP frameworks to develop ecotoxicity prediction models for data-poor plastic additives chemicals, addressing key data gaps in ecological risk assessment. A case study for screening alternative plastic additives is ongoing. This work was supported by Korea Environmental Industry & Technology Institute (KEITI) through 'Core Technology Development Project for Environmental Diseases Prevention and Management', funded by Korea Ministry of Environment (MOE) (2021003310005).

1.06.A.T-04 Revolutionizing Chemical Testing: 70% of Chemicals May Not Require Fish In Vivo Studies

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Environmental risk assessment is required for regulatory approval and marketing of chemicals. It traditionally includes aquatic ecotoxicity studies of species from three trophic levels: algae, crustaceans and fish. The large number of resources, costs, and animals used for toxicity tests, lack of societal acceptance, and phasing out of animal testing for regulatory purposes have resulted in the increasing uptake of the 3Rs (Reduce, Replace, and Refine) principles in safety science. These drivers alongside the push for an enhanced understanding of toxicity, increased the attention towards the use of new approach methodologies (NAMs). One such method is identifying chemicals for which fish are the most sensitive aquatic taxa and understanding the biological mechanisms resulting in toxicity. The ECOTOXicology

Knowledgebase (ECOTOX) with aquatic toxicity data for 10,157 chemicals is a valuable platform for cross-species analysis of toxicity data. We performed a large-scale analysis of the ECOTOX data for comparative sensitivity assessment of different aquatic species to chemicals. We hypothesised that this would help identify group chemicals for which risk assessment based on algae and invertebrate data would be protective of fish, too. We developed a Python pipeline to extract, refine, harmonise, and analyse the median lethal concentrations (expressed as LC50s) and No Observed Effect Concentrations (NOECs) for all available laboratory-based aquatic toxicity studies. Our study shows that most chemicals in the ECOTOX database have comparable median NOECs and LC50 values across different aquatic trophic levels, and fish display higher sensitivity than other species for only a small percentage of chemicals. Understanding the pathways underlying the apparent higher sensitivity of fish to these chemicals may provide a strong rationale for predicting species sensitivity to untested chemicals. It would also bypass the need for chemical testing in fish where a more sensitive lower trophic level is available as an alternative (algae/invertebrate).

1.06.P-Th002 The Impact of Chemicals Found in the Urban Environment on the Genetic Variation in Mice

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Biodiversity has been declining at an alarming rate in recent years due to human activities. Among the pressures exerted in the environment, chemical pollution has been ranked as the third largest driver of biodiversity loss, just below land/sea use change and direct exploitation of natural resources. While chemical risk assessments traditionally focus on species sensitivity to chemical exposure, the impact of chemical pollution on genetic diversity remains underexplored due to challenges such as limited data availability and high costs of sequencing many individuals from wild populations across many species. Nonetheless, the preservation of genetic diversity of wild populations is gathering high interest from the international community and was included in the first global policy agreement at the UN Biodiversity Conference (COP15) in 2022. To better understand how human activities, particularly how chemical pollution can impact the genetic profiles of populations, new computational methods are urgently needed. To address the current knowledge gap of this growing field of research, our approach capitalizes on publicly available data of wild single nucleotide polymorphism (SNPs) from 814 wild mice sampled across 268 locations. We used a landscape genomics approach to characterize the relationship between genetic variation and urban land use data as a proxy for chemical pollution. As a result, we identified 296 genes associated to the urban environment based on significant intragenic SNPs. These genes were mapped to a new, integrated dataset connecting chemicals, toxicity pathways, and phenotypic outcomes based upon public databases (Comparative Toxicogenomics Database, Reactome, Monarch Initiative). Through this, we identified chemicals with strong associations to urban environments, including combustion pollutants, consumer product derivatives, industrial chemicals, and pharmaceuticals. This approach identified genetic variants in genes potentially adapted to the selective pressures exerted by urban chemical exposure. Our framework provides a novel method for prioritizing chemicals based on their potential impacts on the genetics of wild populations, therefore addressing the key challenge of limited data availability. By integrating and repurposing disconnected datasets, this strategy supports conservation goals and offers a scalable tool for chemical risk assessment across species and environments.

1.06.P-Th009 Towards Reliable Chemical Toxicity Predictions with Quantified Uncertainty

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Chemical impact assessments are limited by insufficient data on chemical fate, exposure and effects. Machine learning (ML)-based in silico prediction tools offer high prediction performance and broader applicability than conventional quantitative structure-activity/property relationships. However, their widespread uptake has been hindered by inconsistent applicability across chemicals and lack of transparency regarding model reliability, which undermines confidence in their predictions and complicates integrating predicted and measured data sources. This work demonstrates how uncertainty-aware ML can address some of these challenges by (1) developing models that quantify data- and model-related uncertainty, and (2) using these models to predict reproductive/developmental and general non-cancer human toxicity points of departure (PODs) for >130,000 marketed chemicals. Our results show that the uncertainty-aware ML methods provide well-calibrated uncertainty estimates that are closely aligned with observed prediction errors, assigning more unfamiliar chemicals higher prediction uncertainty. Applying the models to diverse marketed chemicals identifies hotspots of high toxicity, including dioxins

and PFAS, and areas of high uncertainty, such as alkaloids, metals and organometallic compounds. Our results address critical data gaps by providing human toxicity PODs with 95% confidence intervals for a large set of marketed chemicals. Furthermore, they demonstrate how uncertainty-aware ML highlights unreliable chemical classes that should be systematically targeted in future data generation and model development to improve prediction reliability, addressing key drivers of uncertainty such as lack of representation or limited descriptor applicability. Widespread adoption of uncertainty-aware ML will be crucial to enhance the transparency and reliability of ML predictions, paving the way for broader acceptance and application of in silico approaches in chemical assessments. This work was financially supported by the Safe and Efficient Chemistry by Design (SafeChem) project funded by the Swedish Foundation for Strategic Environmental Research (grant no. DIA 2018/11).

1.06.P-Th011 Generalized Multivariable Linear Regression-PBPK Modelling for Nanoparticle Biodistribution Prediction Using Physicochemical Properties

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Physiologically based pharmacokinetic (PBPK) models are essential for predicting nanoparticle (NP) biodistribution, supporting drug delivery and toxicity research. However, traditional PBPK models are often limited to specific NP types, typically requiring animal studies for accurate parameterization, which restricts broader applicability. This study aims to develop a generalized PBPK model that integrates physicochemical properties for early screening of NP delivery efficiency, reducing reliance on animal testing. We developed a generalized PBPK model based on in vivo mouse studies, assisted by multivariable linear regression (MLR) modeling to predict the biodistribution of NPs based on their physicochemical properties. Integrating biodistribution data from five non-dissolvable NPs (Au, FeO, TiO₂, Graphene oxide, and SiO₂), we investigated how NP properties (core material, coating, zeta potential, shape, hydrodynamic size, and dose) affect NP distribution across four key organs (kidney, liver, spleen, lung). The model demonstrated strong predictive performance for kinetic indicators, achieving an adjusted R² of up to 0.95. Predictor frequency analysis indicated that zeta potential and coating had the biggest influence on biodistribution, with NP size also significantly impacting organ-specific interactions. The model accurately predicted delivery efficiency but encountered limitations in estimating the full set of PBPK model parameters, likely due to dataset constraints. For example, some FeO and GO studies with identical NP properties exhibited substantial variance in PBPK model parameter distributions, highlighting the need for more diverse NP property data to enhance predictive accuracy. In conclusion, this generalized MLR-PBPK model provides a practical and interpretable approach for NP evaluation, supporting early screening and reducing the need for animal studies. Expanding the dataset and exploring non-linear models could improve predictions, particularly for full concentration-time profiles. This model represents a promising tool for predictive toxicology and optimized NP design, with potential applicability across species, contributing to safer and more effective NP applications.

1.06.B Data-Driven Toxicology: Practical Applications and Insights into Using Existing and Emerging Data in Support of Human and Environmental Health

1.06.B.T-01 Searching for the Evolutionary Origins of Chemical Toxicity

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Regulatory toxicology faces the fundamental challenge of setting chemical exposure safety limits for many species, including humans, based on experimental data obtained from only a few standard test species. This pragmatic approach to testing representative species of the animal kingdom cannot achieve the desired protection of human health and the environment without an evolutionary and ecological interpretation of why the toxicity experimentally observed in a suite of species (the source of toxicological information) is predictive of the response of other distantly related organisms. PrecisionTox provides evidence on the feasibility of understanding the health impact of chemicals on all animals, including ecological keystone species and humans, based on the evolutionary conservation of pathways to toxicity. The project creates and assembles transcriptomics and metabolomics data into putative biomolecular key toxicity events shared among model species (*Danio rerio* embryos, *Xenopus laevis* embryos, *Daphnia magna*, *Caenorhabditis elegans* and *Drosophila melanogaster*) and human cells. A customised data analysis and visualisation tool allows users to explore the comparative toxicology results from a pilot study to reveal the extent to which specific mechanistic responses in species distributed among long branches of animal phylogeny are interpretable in light of human biology. Overall, the concept of toxicity by descent, which posits that there is an evolutionarily shared response to chemicals across animals, can already be gleaned from a relatively small number of tested substances. PrecisionTox builds a case for One Health Toxicology, allowing for a concerted effort to protect people and the environment from the

harmful effects of chemical pollution. This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 965406.

1.06.B.T-02 A Harmonized ECOTOX Dataset to Train a Random Forest Model for Predicting Ecotoxicity Effects in Honeybees

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Available data for chemical ecotoxicity in honeybee are primarily ED50 acute data. However, ED10 chronic exposure levels correspond more to real-world levels and aligned with state-of-the-art life cycle impact assessment methods, but mostly lacking. We address this gap by developing a random forest model trained by a large, comprehensive ecotoxicity-chemical property dataset with 5328 records covering 540 chemicals. The ecotoxicity data were sourced from the ECOTOX database and primarily harmonized through category and unit standardization to achieve data consistency and uniformity. We performed weighted linear regressions on the harmonized dataset to extrapolate endpoints, establishing an integrated ED10-equivalent chronic and acute dataset across different life stages (adult and larva) and exposure types (oral and dermal). These datasets were enriched with chemical properties, e.g., mode of action, molecular descriptors, chemical categories, from multiple sources. The enriched dataset was split into training and test sets using two splitting schemes: totally random, where the dataset was randomly split, and random by chemical, where the chemicals present in the training and test dataset were kept distinct. Both splitting schemes were tested on the model training and prediction, applying cross-validation to avoid overfitting. The totally random-based model showed a performance with root mean square error (RMSE) of 0.78, while a decreasing performance with an RMSE of 1.34 was observed when using the random by chemical-based model. However, we observed improved performance when constraining the dataset by fixing the life stage and effect type, resulting in RMSE between 0.87 and 0.93. This suggests that the random forest model was hindered by the ecotoxicity variability introduced by data attributes. By fixing these attributes, the model achieved a satisfactory performance even with limited data of 309 records. Eventually, we applied the random forest model to predict the ecotoxicity data of selected pesticides. These data were input into an impact assessment model to characterize the impact of the applied pesticide on honeybee. This study provides an integrated ecotoxicity-chemical property dataset suitable for various model training and data analysis. We also trained a random forest model to predict ecotoxicity data for honeybee and evaluated the impact of unknown chemicals on honeybee using the predicted ecotoxicity data. This project is funded by Bayer AG Crop Science Division.

1.06.B.T-03 Modeling the Bigger Picture: Hierarchical Approaches for Integrating Diverse Toxicological Data in Mechanistic Models

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Molecular measurements from high-throughput methods are becoming increasingly available and extend the existing wealth of diverse toxicological data. Jointly integrating such data into mechanistic models can advance chemical risk assessment by increasing the level of biological understanding. However, there is still a lack of understanding and methods for integrating qualitatively and quantitatively different datasets from different experiments into mechanistic models. Two major challenges remain: 1. Datasets are often incomplete due to experimental limitations (data sparsity). 2. Datasets from multiple sources are noisy due to experimental and biological variation (data variability). To fully utilize such data for advancing the understanding of the underlying processes of toxic effects of chemicals and improving predictions of untested substances, the issues of data sparsity and data variability must be addressed. In the presented work, these issues are addressed by applying Bayesian hierarchical modeling to a diverse dataset. As a mechanistic model, we use a molecular TKTD model fitted on time-resolved internal concentration, nrf2 expression, and survival data after exposure to diclofenac, diuron, and naproxen from 42 experiments with zebrafish embryos. To study the potential of hierarchical modeling, we estimate the true external concentration as a function of the nominal external concentration multiplied by an experiment-specific deviation factor. Early results show that, on average, the modeled external concentrations deviate by a factor of 2.1 (94% HDI = [1.9, 2.3]) with a log standard deviation of 0.81 from the nominal concentrations. Using the hierarchical approach, all 42 experiments included in the entire dataset could be used. In contrast, when estimating the model parameters without a hierarchical approach, 11 experiments where exposure concentrations clearly deviated from the nominal concentrations had to be excluded to achieve a reasonable fit. The approach shows how hierarchical modeling approaches can be instrumental in revisiting existing experimental data and integrating them into mechanistic models together with newly

incoming molecular data to understand chemical effects in organisms and approach predictive risk assessment.

1.06.B.T-04 A Data Analysis Pipeline for High-Content Bioimage Phenotyping in Toxicology: Development and Applications

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Our research on fish demonstrated a strong link between in vitro cellular response, such as reduced proliferation, and in vivo impaired growth following chemical exposure. Chemicals that didn't impair growth in fish similarly fail to reduce cell count in vitro. This raises a question: how do chemicals with diverse structures and mechanisms of action result in the same outcome reduced cell population and thus, impaired fish growth?

Investigating these questions, we used the RTgill-W1 cell line from rainbow trout, known for its predictive power in linking in vitro cell numbers with in vivo organism size. We employed established methods, such as chemical exposure experiments and transcriptomics, and innovative methods such as translomics and high-content bioimage phenotyping.

Here, we present a novel computational pipeline for the analysis of high-content bioimages for ecotoxicological purposes. The pipeline includes extracting information from segmented images, quality control with trained classifiers, pooling of the data at the individual cell level, removal of batch effects and outliers, and feature selection and correlation filtering. Exploration of each step allowed us to establish an optimal decision tree for noise reduction and refining the data for interpretation. Among other, we showed that standardization of the data is needed for accurate analysis of the images, while removal of the artefacts seemed less important. We also introduced a new method for selecting the most relevant features based on dose-response considerations; and showed that the maximum-mean discrepancy can be used as a measure of image perturbation, which is slightly more sensitive than traditional cell-count based concentration-response curve fitting. We found that the 2D UMAP data projection was the most helpful for biological interpretation of the results, as it shows a separation of the cell populations both with increasing chemical concentration as well as with days after exposure. Features related to the lysosome and nuclei intensity were among the most perturbed, pointing to impaired autophagy and cell cycle disruption.

Tailored to our specific experimental setup and biological question, this pipeline enabled the management and analysis of large bioimaging datasets, producing clearer dose-response signals for interpreting cellular mechanisms. The presented study serves as a blueprint for constructing similar workflows for a wide range of high-content image-based assays.

1.06.P-Th001 Benchmarking Environmental Risk for Plant Protection Products: A Case for Insecticides and Acaricides

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Agricultural pesticides, while vital for crop protection, are increasingly recognised as critical contributors to biodiversity loss despite stringent regulatory frameworks in the European Union (EU). Current environmental risk assessments (ERA) for plant protection products (PPPs) often fail to provide comparable risk outcomes across substances, taxonomic groups, and environmental compartments. This study addresses this gap by developing a benchmarking framework to rank PPPs based on their relative environmental risks, focusing on insecticides and acaricides approved in the EU. We compiled a comprehensive database using prospective ERA data from regulatory sources to achieve this. Key parameters included toxicity, persistence, bioaccumulation, mobility, and exposure scenarios. The risk quotient (RQ) for aquatic organisms served as the primary benchmark, enabling comparisons across organism groups, representative uses, time scales, tiers, and exposure scenarios.

Preliminary results highlight significant variability in RQs, with several substances approved only for specific uses or with risk mitigation measures. Notably, substances with high persistence and bioaccumulative potential posed elevated risks. These findings underline the importance of tier-specific analyses and the need for consistent, comparative methodologies.

Our study underscores the potential of benchmarking to enhance the transparency and efficiency of ERAs, enabling more informed regulatory decisions. This framework can advance biodiversity protection and inform future regulatory practices by identifying high-risk substances and prioritising mitigation strategies. This investigation is supported by the European Partnership for the Assessment of Risks from Chemicals (PARC) under grant agreement No. 101057014. This publication reflects only the author's

view, and the European Commission is not responsible for any use that may be made of the information it contains.

1.06.P-Th010 Prediction of In Vitro Neurotoxicity Through Machine Learning

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Neurotoxics, i.e., chemicals that specifically affect structural or functional aspects of the nervous system, pose a challenge to environmental hazard assessment. Neurotoxicity can follow different modes of actions by targeting specific receptors, molecules, or cells within an organism. Hence, many in vitro assays have been developed to assess the effects of chemicals on different mechanistic targets or cellular structures. However, even with their marked advantages, high throughput in vitro methods cannot fulfill the growing needs for fast and cost-effective neurotoxicity testing of chemicals. Here, in silico methods, such as machine learning models, can integrate a range of in vitro endpoints to allow for rapid and, after model implementation, low-cost prediction of neurotoxic potential across a broad range of assay endpoints. We selected neurotoxicity-related data for in vitro effect assays from the latest version of invitroDB database and processed them through the pytcpl pipeline by Arturi et al. (under revision). The resulting dataset of 50 endpoints was split into training and test data and used to develop binary classification models (logistic regression, support vector machines, random forest, and extreme gradient boosting (XGBoost)). The Friedman s tests for all endpoints showed that, based on average precision, ROC, and MCC, the models perform similarly, and no model is superior. For 42 of 50 endpoints all models performed better than random and for more than half of the endpoints all models achieved an average precision above 0.6. Class imbalance, e.g., overrepresentation of inactive compounds, and differences in the range of chemical properties between training and test set influenced performance as well as the threshold used to label a chemical as active in a respective endpoint. The latter was further investigated at different levels (response levels of 0.1, 0.5, 0.9 in an endpoints) and, for some endpoints, lowering the threshold towards the more conservative level of 0.1 improved the performance. Overall, the application of machine learning models to predict neurotoxicity based on in vitro data shows promise to enable rapid prediction of the neurotoxic potential of chemicals. This, in turn, strengthens the case for the use of machine learning to integrate data sources from different alternatives to animal testing with chemical features to cover a broad spectrum of modes of action.

1.06.P Data-Driven Toxicology: Practical Applications and Insights into Using Existing and Emerging Data in Support of Human and Environmental Health

1.06.P-Th001 Benchmarking Environmental Risk for Plant Protection Products: A Case for Insecticides and Acaricides

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Agricultural pesticides, while vital for crop protection, are increasingly recognised as critical contributors to biodiversity loss despite stringent regulatory frameworks in the European Union (EU). Current environmental risk assessments (ERA) for plant protection products (PPPs) often fail to provide comparable risk outcomes across substances, taxonomic groups, and environmental compartments. This study addresses this gap by developing a benchmarking framework to rank PPPs based on their relative environmental risks, focusing on insecticides and acaricides approved in the EU. We compiled a comprehensive database using prospective ERA data from regulatory sources to achieve this. Key parameters included toxicity, persistence, bioaccumulation, mobility, and exposure scenarios. The risk quotient (RQ) for aquatic organisms served as the primary benchmark, enabling comparisons across organism groups, representative uses, time scales, tiers, and exposure scenarios.

Preliminary results highlight significant variability in RQs, with several substances approved only for specific uses or with risk mitigation measures. Notably, substances with high persistence and bioaccumulative potential posed elevated risks. These findings underline the importance of tier-specific analyses and the need for consistent, comparative methodologies.

Our study underscores the potential of benchmarking to enhance the transparency and efficiency of ERAs, enabling more informed regulatory decisions. This framework can advance biodiversity protection and inform future regulatory practices by identifying high-risk substances and prioritising mitigation strategies. This investigation is supported by the European Partnership for the Assessment of Risks from Chemicals (PARC) under grant agreement No. 101057014. This publication reflects only the author's view, and the European Commission is not responsible for any use that may be made of the information it contains.

1.06.P-Th002 The Impact of Chemicals Found in the Urban Environment on the Genetic Variation in Mice

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Biodiversity has been declining at an alarming rate in recent years due to human activities. Among the pressures exerted in the environment, chemical pollution has been ranked as the third largest driver of biodiversity loss, just below land/sea use change and direct exploitation of natural resources. While chemical risk assessments traditionally focus on species sensitivity to chemical exposure, the impact of chemical pollution on genetic diversity remains underexplored due to challenges such as limited data availability and high costs of sequencing many individuals from wild populations across many species. Nonetheless, the preservation of genetic diversity of wild populations is gathering high interest from the international community and was included in the first global policy agreement at the UN Biodiversity Conference (COP15) in 2022. To better understand how human activities, particularly how chemical pollution can impact the genetic profiles of populations, new computational methods are urgently needed. To address the current knowledge gap of this growing field of research, our approach capitalizes on publicly available data of wild single nucleotide polymorphism (SNPs) from 814 wild mice sampled across 268 locations. We used a landscape genomics approach to characterize the relationship between genetic variation and urban land use data as a proxy for chemical pollution. As a result, we identified 296 genes associated to the urban environment based on significant intragenic SNPs. These genes were mapped to a new, integrated dataset connecting chemicals, toxicity pathways, and phenotypic outcomes based upon public databases (Comparative Toxicogenomics Database, Reactome, Monarch Initiative). Through this, we identified chemicals with strong associations to urban environments, including combustion pollutants, consumer product derivatives, industrial chemicals, and pharmaceuticals. This approach identified genetic variants in genes potentially adapted to the selective pressures exerted by urban chemical exposure. Our framework provides a novel method for prioritizing chemicals based on their potential impacts on the genetics of wild populations, therefore addressing the key challenge of limited data availability. By integrating and repurposing disconnected datasets, this strategy supports conservation goals and offers a scalable tool for chemical risk assessment across species and environments.

1.06.P-Th003 Data-Driven Insights into Plastic Additives-Induced Toxicities Using Stressor-centric Adverse Outcome Pathway (AOP) Networks

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Plastics are pervasive pollutants in atmospheric, terrestrial, and aquatic ecosystems due to their widespread use and environmental persistence. Plastic additives, intentionally incorporated to enhance plastic properties, leach into the environment upon plastic degradation, posing significant ecological and human health risks. Limited knowledge of these chemicals across the plastic life cycle hampers effective regulation and product safety assessments. To address this challenge, a data-centric approach was employed to investigate the toxicological impacts of plastic additives through the Adverse Outcome Pathway (AOP) framework. The study also focused on classification of additives by their presence in priority use sectors, exploration of associated toxicity pathways, and identification of prevalent adverse health outcomes linked to plastic additives. A dataset of 6470 additives from chemicals documented in plastics was curated and classified into ten priority use sectors, including food packaging, personal care products, and agriculture. Toxicogenomics and biological endpoint data from five exposome-relevant resources were integrated to identify associations between 1287 additives and 322 complete, high-quality AOPs documented in AOP-Wiki. These associations informed the construction of a stressor-centric AOP network, categorizing additives by use sectors and organizing AOPs under 27 disease categories according to the documented adverse outcomes. Individual plastic additive AOP networks were visualized and made publicly accessible at <https://cb.imsc.res.in/saopadditives/>. The utility of the constructed plastic additives AOP network was demonstrated by identifying highly relevant AOPs for three priority additives: benzo[a]pyrene (B[a]P), bisphenol A (BPA), and bis(2-ethylhexyl) phthalate (DEHP). Published experimental evidence was used to explore the human- and ecotoxicology-relevant toxicity pathways associated with these additives. Additionally, this study revealed that many of these additives, which are produced in high volumes globally, can potentially accumulate in human tissues as xenobiotics, and lead to severe adverse effects on various organ systems. Overall, this study provides the first stressor AOP network for plastic additives, revealing toxicity pathways and prevalent adverse outcomes. It also assists

in their risk assessment and regulatory decision-making, thereby contributing towards a toxic-free circular economy for plastics.

1.06.P-Th004 Development of an Adverse Outcome Pathway Leading to Neurodevelopmental Disorders and its Application to Screening Chemicals in Consumer Products

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The environmental causes of neurodevelopmental disorders (NDDs) are still poorly understood. There is growing evidence that exposure to various chemicals in consumer products, including endocrine disrupting chemicals, potentially affect development and behavior. In this context, a putative AOP network associated with NDD was identified to provide insight into the NDD potential of chemicals in consumer products using an Integrated Testing Strategy (ITS). First, human genes related to the synaptic function mechanism of NDD were selected using the SFARI Gene database. Next, the putative AOP network was developed using the database mining approach using the Comparative Toxicogenomics Database and AOP Wiki. Leveraging this AOP network, chemicals in consumer products were screened by assigning ToxCast assays to specific key events (KEs). Further experiments were performed on KEs that were not assigned to the ToxCast assay, such as BDNF level, NMDA receptor binding, and learning (measured as development and behavior). For these assays, human embryonic stem cells, human neural stem cells, *Caenorhabditis elegans* wildtype and mutants (*nlg-1(lf)* and *nrx-1(lf)*) were exposed to chemicals and molecular docking were performed. Among the chemicals in consumer products, BPA and DEHP showed high bioactivity for ToxCast assay related to AOP network such as calcium influx, decreased synaptogenesis, and decreased neuronal network function. Next, BPA has been predicted to have the highest binding affinity for NMDA receptor binding, a Molecular Initiating Event. When exposed to BPA and DEHP, *C. elegans* (wild type, *nlg-1*, *nrx-1*) showed significant changes in locomotive behavior and BDNF levels in human stem cells were also altered. BPA has the highest NDD-leading potential, as BPA reacted with most KEs. ITS was applied to ToxCast database, in silico prediction, in vitro, stem cells, and *C. elegans* disease models. This study suggests that environmentally concerning chemicals can be screened for their effects on disease potential using our ITS. This work was supported by Korea Environment Industry & Technology Institute (KEITI) through Technology Development Project for Safety Management of Household Chemical Products, funded by Korea Ministry of Environment (MOE)(RS-2023-00215309).

1.06.P-Th005 Leveraging Zebrafish Embryo Phenotypic Observations to Advance Data-Driven Analyses in Toxicology

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Zebrafish have emerged as a central model organism in toxicological research. Zebrafish embryos are exempt from certain animal testing regulations which facilitates their use in toxicological testing. Next to the zebrafish embryo acute toxicity test (ZFET) according to the OECD TG 236, fish embryos are used in mechanistic investigations, chemical screenings, in ecotoxicology, and drug development. However, inconsistencies in the applied test protocols and the monitored endpoints in addition to a lack of standardized data formats, impede comprehensive meta-analyses and cross-study comparisons. To address these challenges, we developed the Integrated Effect Database for Toxicological Observations (INTOB), a comprehensive data management tool that standardizes collection of metadata and phenotypic observations using a controlled vocabulary. By incorporating data from more than 600 experiments into the database and subsequent comprehensive data analyses, we demonstrate its utility in improving the comparability and interoperability of toxicity data. Our results show that the ZFET can detect toxicity spanning seven orders of magnitude at the scale of effect concentrations. We also highlight the potential of read-across analyses based on morphological fingerprints and their connection to chemical modes of action, provide information on control variability of the ZFET, and highlight the importance of time for mechanistic understanding in chemical exposure-effect assessments. With our data analysis workflow and INTOB database we demonstrate how professional data management marks a significant advancement by offering a comprehensive framework for the systematic use of zebrafish embryo toxicity data, thus paving the way for more reliable, data-driven chemical risk assessment.

1.06.P-Th006 Derivation of Hazardous Concentrations of Soil Pollutants for The TRIAD Approach

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The TRIAD approach, published by ISO in 2017 for site-specific soil ecological risk assessment, integrates chemical-, ecotoxicological-, and ecological-lines of evidence (LoEs) to calculate integrated risk (IR). To determine and refine the chemical-LoE, hazardous concentrations based on species sensitivity distribution (SSD) require toxicity data on pollutants. Some countries (e.g., USA, Australia, Netherlands, UK, and Canada) has their own ecological protection standards, meanwhile, other countries may not have their own standards for pollutants. Thus, this study aimed to collect soil toxicity data of soil pollutants and to estimate hazardous concentrations based on SSD for TRIAD. Twenty pollutants were prioritized and categorized, and their toxicity data were sourced and classified from the USEPA ECOTOX database. Hazardous concentrations were then estimated based on SSD analysis. This study is significant as it redefines ecological protection standards for soil pollutants using diverse data sources, making the results applicable to international guidelines, irrespective of specific national standards. Our data provide a foundation for site-specific ecological risk assessments, contributing to the development and enhancement of the TRIAD guideline. This work was supported by Korea Environment Industry & Technology Institute (KEITI) funded by Korea Ministry of Environment No.2022002450002(RS-2022-KE002074).

1.06.P-Th007 Network-Based Investigation of Petroleum Hydrocarbons-Induced Ecotoxicological Effects and Their Risk Assessment

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Petroleum hydrocarbons (PHs), primarily made of carbon and hydrogen, are significant environmental pollutants derived from crude oil and its products. They are introduced into ecosystems through anthropogenic activities such as transportation, offshore drilling, and oil spills, causing long-term ecological harm. While environmental risks of PHs are often assessed using total petroleum hydrocarbon (TPH) levels, the risks posed by individual PHs and their toxicity mechanisms remain underexplored. This study addresses this challenge by leveraging network-based approaches to evaluate the ecological effects of PH exposure. A curated dataset of 320 PHs was developed from literature, and toxicological endpoint data were systematically integrated. Stressor-centric adverse outcome pathway (AOP) networks linked 75 PHs to 177 ecotoxicologically relevant AOPs in AOP-Wiki, while stressor-species networks were constructed using toxicity concentration and bioconcentration data from ECOTOX for 80 and 28 PHs, respectively. Species sensitivity distributions (SSDs) were generated to calculate hazard concentrations (HC05) for aquatic species and predicted no-effect concentrations (PNECs) and risk quotients were derived for US EPA priority polycyclic aromatic hydrocarbons (PAHs) based on environmental data from Indian coastal and river waters. Notably, these analyses revealed that many aquatic species, particularly crustaceans, are highly sensitive to PH exposure, emphasizing the need for targeted protective measures in ecosystems where such pollutants are prevalent. This study advances the understanding of PH-induced toxicity through network-based approaches, uncovering PH-specific toxicity pathways and ecological risks. Additionally, derived threshold concentrations and AOP data can guide environmental quality standards. Findings have practical applications for events like oil spills, enabling the formulation of more effective mitigation strategies. By using network-based frameworks to analyze ecotoxicological data, this study highlights how PHs affect ecological species, with potentially broader implications for human and animal health within the One Health context. Ultimately, this study highlights the importance of regulating PHs in the environment and safeguarding ecosystem health.

1.06.P-Th008 Enhancing Interpretability of High-Throughput Toxicological Data Using Self-Organising Maps

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The availability of toxicity data for the huge number of chemicals found in the environment is limited, which results in substantial challenges during monitoring and assessment of environmental contamination. High-throughput initiatives like the Tox21 program of the US-Environmental Protection Agency (EPA) aim to fill this gap by supplying in vitro effect data for thousands of chemicals and hundreds of different endpoints. Yet, even such datasets can be scarce and heterogeneous due to, for instance, varying test

substances and concentration ranges, which often limits the predictive power of models based on this data. In order to address these challenges, the hypothesis that self-organising maps (SOMs) can be employed to enhance the interpretability of such effect data and to fill data gaps was formulated. Therefore, data from the ToxCast database (version 4.1, US-EPA) were manually curated and aggregated. Concentration-response curves of reference substances were modelled using nonlinear Bayesian regression. Subsequently, effect data of test substances were normalised based on these reference curves of the respective assay followed by modelling the concentration-response curves of test substances using nonlinear least-squares regression. The respective best-fitting models were used for the extrapolation of effect data within a defined concentration range. Finally, using the SOM algorithm, assays were organised into a two-dimensional map based on the similarity of the extrapolated effect curves of the tested substances, thereby reducing data dimension and filling data gaps. The SOM performance was enhanced by tuning hyperparameters based on summed squares of topological error and explained variance. Bootstrap resampling with the tuned parameters was performed to analyse robustness. The trained SOM can be used, for instance, in the assessment of environmental monitoring data of chemical contamination for screening and prioritisation of chemicals and their effects. To do so, effect profiles for specific chemicals or mixtures using the map's structure were generated exemplarily as a concise visual representation of in vitro effects. In summary, a framework is proposed to complement gap-filling approaches in an effect-based way rather than based on chemical structure as in read-across approaches, while also increasing the robustness and interpretability of effect data, thereby aiding in prioritising chemicals and effects in environmental monitoring.

1.06.P-Th009 Towards Reliable Chemical Toxicity Predictions with Quantified Uncertainty

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Chemical impact assessments are limited by insufficient data on chemical fate, exposure and effects. Machine learning (ML)-based in silico prediction tools offer high prediction performance and broader applicability than conventional quantitative structure-activity/property relationships. However, their widespread uptake has been hindered by inconsistent applicability across chemicals and lack of transparency regarding model reliability, which undermines confidence in their predictions and complicates integrating predicted and measured data sources. This work demonstrates how uncertainty-aware ML can address some of these challenges by (1) developing models that quantify data- and model-related uncertainty, and (2) using these models to predict reproductive/developmental and general non-cancer human toxicity points of departure (PODs) for >130,000 marketed chemicals. Our results show that the uncertainty-aware ML methods provide well-calibrated uncertainty estimates that are closely aligned with observed prediction errors, assigning more unfamiliar chemicals higher prediction uncertainty. Applying the models to diverse marketed chemicals identifies hotspots of high toxicity, including dioxins and PFAS, and areas of high uncertainty, such as alkaloids, metals and organometallic compounds. Our results address critical data gaps by providing human toxicity PODs with 95% confidence intervals for a large set of marketed chemicals. Furthermore, they demonstrate how uncertainty-aware ML highlights unreliable chemical classes that should be systematically targeted in future data generation and model development to improve prediction reliability, addressing key drivers of uncertainty such as lack of representation or limited descriptor applicability. Widespread adoption of uncertainty-aware ML will be crucial to enhance the transparency and reliability of ML predictions, paving the way for broader acceptance and application of in silico approaches in chemical assessments. This work was financially supported by the Safe and Efficient Chemistry by Design (SafeChem) project funded by the Swedish Foundation for Strategic Environmental Research (grant no. DIA 2018/11).

1.06.P-Th010 Prediction of In Vitro Neurotoxicity Through Machine Learning

Christoph Schuer¹, Lilian Gasser², Antoine Escoyez², Kristin Schirmer¹, Guillaume Obozinski² and Marco Baity-Jesi¹, (1)Eawag, Swiss Federal Institute of Aquatic Science and Technology, Switzerland, (2)Swiss Data Science Center (SDSC), Switzerland
Neurotoxicants, i.e., chemicals that specifically affect structural or functional aspects of the nervous system, pose a challenge to environmental hazard assessment. Neurotoxicity can follow different modes of actions by targeting specific receptors, molecules, or cells within an organism. Hence, many in vitro assays have been developed to assess the effects of chemicals on different mechanistic targets or cellular structures. However, even with their marked advantages, high throughput in vitro methods cannot fulfill the growing needs for fast and cost-effective neurotoxicity testing of chemicals. Here, in silico methods, such as machine learning models, can integrate a range of in vitro endpoints to allow for rapid and, after model implementation, low-cost prediction of neurotoxic potential across a broad range of assay

endpoints. We selected neurotoxicity-related data for in vitro effect assays from the latest version of invitroDB database and processed them through the pytcpl pipeline by Arturi et al. (under revision). The resulting dataset of 50 endpoints was split into training and test data and used to develop binary classification models (logistic regression, support vector machines, random forest, and extreme gradient boosting (XGBoost)). The Friedman s tests for all endpoints showed that, based on average precision, ROC, and MCC, the models perform similarly, and no model is superior. For 42 of 50 endpoints all models performed better than random and for more than half of the endpoints all models achieved an average precision above 0.6. Class imbalance, e.g., overrepresentation of inactive compounds, and differences in the range of chemical properties between training and test set influenced performance as well as the threshold used to label a chemical as active in a respective endpoint. The latter was further investigated at different levels (response levels of 0.1, 0.5, 0.9 in an endpoints) and, for some endpoints, lowering the threshold towards the more conservative level of 0.1 improved the performance. Overall, the application of machine learning models to predict neurotoxicity based on in vitro data shows promise to enable rapid prediction of the neurotoxic potential of chemicals. This, in turn, strengthens the case for the use of machine learning to integrate data sources from different alternatives to animal testing with chemical features to cover a broad spectrum of modes of action.

1.06.P-Th011 Generalized Multivariable Linear Regression-PBPK Modelling for Nanoparticle Biodistribution Prediction Using Physicochemical Properties

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Physiologically based pharmacokinetic (PBPK) models are essential for predicting nanoparticle (NP) biodistribution, supporting drug delivery and toxicity research. However, traditional PBPK models are often limited to specific NP types, typically requiring animal studies for accurate parameterization, which restricts broader applicability. This study aims to develop a generalized PBPK model that integrates physicochemical properties for early screening of NP delivery efficiency, reducing reliance on animal testing. We developed a generalized PBPK model based on in vivo mouse studies, assisted by multivariable linear regression (MLR) modeling to predict the biodistribution of NPs based on their physicochemical properties. Integrating biodistribution data from five non-dissolvable NPs (Au, FeO, TiO₂, Graphene oxide, and SiO₂), we investigated how NP properties (core material, coating, zeta potential, shape, hydrodynamic size, and dose) affect NP distribution across four key organs (kidney, liver, spleen, lung). The model demonstrated strong predictive performance for kinetic indicators, achieving an adjusted R² of up to 0.95. Predictor frequency analysis indicated that zeta potential and coating had the biggest influence on biodistribution, with NP size also significantly impacting organ-specific interactions. The model accurately predicted delivery efficiency but encountered limitations in estimating the full set of PBPK model parameters, likely due to dataset constraints. For example, some FeO and GO studies with identical NP properties exhibited substantial variance in PBPK model parameter distributions, highlighting the need for more diverse NP property data to enhance predictive accuracy. In conclusion, this generalized MLR-PBPK model provides a practical and interpretable approach for NP evaluation, supporting early screening and reducing the need for animal studies. Expanding the dataset and exploring non-linear models could improve predictions, particularly for full concentration-time profiles. This model represents a promising tool for predictive toxicology and optimized NP design, with potential applicability across species, contributing to safer and more effective NP applications.

1.06.P-Th012 Evaluation of Human Exposure to Phthalates and Correlation with Contamination in the Indoor Environment

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The technological advances in the last century have led to the large scale production of a huge number of synthetic organic compounds, used in various types of applications. As a result, high concentrations of toxic substances are now present in the environment, which can have harmful effects on human health and ecosystems. One group of compounds that has raised great concern is the phthalates, which, despite the imposition of restrictions and regulations in some countries, still reach an annual global consumption of 6 million tons. These products are mainly employed as plasticizers, which are used in the production of food packaging, toys, cosmetics, medical devices, and construction materials, among others. Phthalates are not covalently bound to polymers and can easily migrate into the environment or fluid system, being commonly reported in environmental samples, food and inside environments. Knowing that a large part of post-pandemic society has adopted hybrid working hours, which means that they stay in their homes for longer periods each week, the presence of these compounds in indoor air and dust samples has been indicated as an important route of human exposure to phthalates, through involuntary ingestion, inhalation, and/or dermal absorption. Human exposure to these products has been proven to be related to various

diseases and health implications. Thus, the aim of this study was to reuse existing data in the scientific literature on the occurrence of phthalate products inside homes, considering air and dust samples, as well as their correlation with biological samples from residents of the habitations. For this, we analyzed empirical data and used equations to estimate the daily uptake per individual and the health risk associated with these pollutants. Our results indicate that the phthalate metabolites identified through biomonitoring correlate with the phthalates identified in the internal environment of the homes, with higher molecular weight phthalates being more frequent in dust samples and lower molecular weight phthalates being more frequent in air samples. The risk assessment shows that children are the most vulnerable to daily intake through involuntary ingestion of dust, and the estimated values are worrying and exceed the tolerable limits set by international authorities. For further information and risk assessments of human exposure in this context, more studies covering the African continent and South America are still needed. We thank the Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Universidade Estadual de Campinas (UNICAMP) for supporting our research.

1.06.P-Th013 Improving the Reliability of Chemical Property Predictions: A Thermodynamic Perspective

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Accurate prediction of physical and chemical properties is crucial for assessing the dispersion and fate of chemicals in a multimedia environment. These properties are often estimated by Quantitative Structure-Activity or Structure-Property Relationships (QSAR/QSPR), which are trained on large sets of experimental data with molecular structure as independent variables. These models are often trained to directly calculate the desired physicochemical properties. In this case, the consistency of the estimated values relies on the consistency of the training data and the potential of the models to capture the thermodynamic relationships between the predicted properties. An alternative is to first obtain experimental values or estimate the critical properties of the pure chemicals with a QSPR method (e.g. Joback and its extensions) and then use an appropriate mechanistic or semiempirical model, e.g. an equation of state to estimate the properties. This method ensures the consistency of the estimated properties but does not guarantee their accuracy. Here, we utilize both workflows to estimate the physicochemical properties of a large database of compounds for which some of the physicochemical properties and critical properties are measured. For the QSAR/QSPR method, we use EPI Suite and OPERA for estimating pure chemical (vapor/sublimation pressure, boiling/melting point) and multiphase binary/ternary properties (water solubility, Henry's law constant, and octanol-water partition coefficient). We repeat the calculations with semi-empirical thermodynamic relations, cubic equations of state and predictive activity coefficient model UNIFAC in combination with the experimental and estimated critical properties. We finally compare the estimations with available experimental data and flag the experimental values that are inconsistent with the rest of the data, and rank the models based on the closest prediction to the experimental data for each compound. Finally, we utilize the highest ranked model to predict the physicochemical properties of ~10,000 compounds that are included in the next release of USEtox 3. By systematically evaluating the thermodynamic consistency of QSAR/QSPR predictions and training data, we aim to improve the accuracy and reliability of chemical fate and transport models, ultimately leading to more informed environmental risk assessments.

1.06.P-Th014 Critical Review of the Environmental Exposure and Ecotoxicity Potential of Decabromodiphenylethane (DBDPE) in Aquatic and Terrestrial Biota Using a Chemical Activity-Based Risk Assessment

Kate Fremlin, Nicole Berg and Frank Gobas, Simon Fraser University, Canada

Screening assessments of decabromodiphenylethane (DBDPE) concluded that DBDPE was persistent but non-toxic at concentrations below its measured aqueous solubility of 0.72 µg/L. However, the assessments were inconclusive on the bioaccumulation potential of DBDPE and whether the current concentrations detected in the environment have potential to bioaccumulate to levels of concern. To address this knowledge gap, we performed a risk assessment and reviewed 66 studies that reported concentrations of DBDPE measured in a range of plants and animals and 58 studies that reported toxic effect concentrations of DBDPE determined in toxicity tests. The objectives of the risk assessment were 1) to critically evaluate all the studies that measured concentrations of DBDPE in biota with a set of comprehensive criteria and guidelines for conducting field studies that monitor chemical concentrations in the environment; and 2) to use a weight of evidence analysis to facilitate the comparison between the environmental exposure concentrations and the toxic effect concentrations. To perform the risk assessment, we used a chemical activity analysis of the available exposure and toxicity data for DBDPE. Approximately 70% of the studies that reported concentrations of DBDPE measured in biota were considered of acceptable quality whereas 30% of the studies were considered of unacceptable quality so concentrations from these studies

were excluded from further analyses. The chemical activity analysis showed that to date all toxicological effects endpoints of DBDPE in toxicity tests occurred at concentrations that are equal to chemical activities of DBDPE that are well above the maximum chemical activity of DBDPE. Essentially, DBDPE concentrations used in toxicological tests were above the sorptive capacity of the medium involved in the toxicity tests and are not thermodynamically feasible to occur in the environment. Conversely, the chemical activity analysis showed that concentrations of DBDPE measured in biota from various locations around the world were below the maximum chemical activity of DBDPE so are considered thermodynamically feasible. Moreover, the chemical activities of DBDPE in biota from around the world are several orders of magnitude lower than the chemical activities of DBDPE that are associated with toxicological effects of DBDPE. Thus, to date DBDPE has not accumulated in biota to levels of toxicological concern or which present a risk for adverse effects.

1.06.P-Th015 In-silico Models for the Toxicity Prediction of Disinfection By-Products (DBPs) Resulting from Disinfection Processes in Drinking Water Treatment Plants (DWTPs)

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According to the European Environmental Agency (EEA), most of the EU's freshwater use comes from rivers and ground water. Besides, the European Water Report of 2018, showed that around 60% of European surface waters are not in good ecological status and 62% are not in good chemical status. With this information, it can be appreciated a risk of humans being exposed to different pollutants. In terms of contamination of drinking water there are some challenges that drinking water treatment plants (DWTPs), last barrier before human exposure, must face, such as the hazardous disinfection processes, which lead to the formation of different products known as Disinfection By-Products (DBPs), which come from the natural organic matter present in water. Although many DBPs have already been identified, most remain unregulated. It is considered that the understanding of their formation mechanisms as well as the study of their toxicity need to be evaluated. For this, in-silico models which aim to predict these characteristics are being developed. Firstly, a specific model to predict the final DBPs for four pollutants is being implemented. In addition, two Quantitative Structure Activity Relationship (QSAR) models to predict carcinogenicity and genotoxicity are being developed. With these models, it would be possible to estimate carcinogenicity and genotoxicity for predicted DBPs and filter the most representative ones to further evaluate the actual toxicity using in vitro assays to deepen understanding of their effects. Particularly valuable will be assays utilizing cell lines that represent major human exposure routes. To ensure the reliability of the QSAR models, two main methods have been used: statistical validation to quantify how closely they align with actual data and similarity tests to ensure predicted molecules remain within the applicability domain. This validation shows low accuracy for both classification model (genotoxicity) and regression one (carcinogenicity) showed low accuracy for prediction. To improve the performance, we are working on retraining both models to further exploration of the parameter space.

This integrated approach, combining predictive modelling with in vitro tailored to OECD guidelines, exemplifies a New Approach Methodology (NAM) for chemical risk assessment. It provides a more detailed understanding of the carcinogenic and genotoxic effects of chemicals through integrative analysis of new sources and types of data. Abstract written thanks to funding from the European Union, within the Horizon Europe programme under grant agreement 101082015.

1.06.P-Th016 Aquatic Toxicity of Palladium: A Grouping and Read-Across Approach for Some Palladium Substances and Why Others Need Separate Assessments

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Palladium (Pd) is crucial in catalytic converters, significantly reducing greenhouse gas emissions. However, its use has increased Pd concentrations in freshwater, where its solubility enhances mobility and bioavailability, potentially harming aquatic organisms. Despite this, the ecotoxicity database for Pd is limited. This study summarizes acute and chronic ecotoxicity data for algae, invertebrates, and fish, developing a grouping and read-across approach to derive threshold concentrations. Additionally, it identifies substances requiring individual assessment due to unique properties. The findings aim to support hazard and risk assessments in an EU regulatory context. A literature review and quality assessment were conducted to identify reliable data, supplemented by industry-owned test data. Ecotoxicity thresholds were derived as lethal/ effective concentration 50 (L/EC50), causing death/ sublethal effects in 50% of organisms for acute studies, and as no observed effect concentration (NOEC) or effective concentration 10 (EC10) for chronic studies. Using aquatic speciation, toxicity data, and chemical structure, a grouping approach was proposed for hazard assessment. Reliable acute data are available for algae, invertebrates, and fish, while chronic data exist only for algae and invertebrates. Algae emerged as the most sensitive trophic level in both study types, with a sensitivity gradient of algae > invertebrates > fish. Acute

thresholds for algae align with predictions from the quantitative ion-activity relationship (QICAR) model. Toxic thresholds for all species remain significantly above natural Pd levels ($<0.001 \mu\text{g Pd/L}$). Most simple inorganic Pd substances respeciate to palladium(II) hydroxide upon dissolution, with negligible effects from counterions. These substances were grouped for read-across assessment. However, compounds like palladium di(4-oxopent-2-en-2-oate), palladium(II) acetate, and palladium dinitrate showed unique speciation or toxicity profiles. For example, palladium di(4-oxopent-2-en-2-oate) exhibited an atypical sensitivity gradient (fish > algae > invertebrates), while palladium dinitrate showed lower toxicity across all trophic levels. These substances require individual assessments. This study validates the read-across approach for simple Pd substances, highlighting algae as the most sensitive trophic level. However, it emphasizes that certain Pd compounds, due to their distinct properties, must be assessed individually.

1.06.P-Th017 Applying In Vitro High-Throughput and High-Content Screening Assays for Toxicity Analysis of Environmental Water Samples: Emphasis on Liver and Endocrine Disrupting Effects

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Rapid global population growth and increasing human activities have driven industrial development, leading to excessive pollutant emissions into aquatic environments. This has severely contaminated vital water resources, particularly lakes, rivers, and oceans, threatening marine life and potentially affecting human health through the food chain. Therefore, monitoring water quality is critical to ensuring public safety. Conventional chemical analysis typically focuses on individual substances and may underestimate the toxicity of environmental water, as it cannot detect the biological effects of mixtures of pollutants. In contrast, integrating biological analysis methods provides a faster and more sensitive approach that detects both individual compounds and complex pollutants, allowing for a more comprehensive understanding of environmental water quality. This study collected river water and industrial wastewater samples from various sites in Taoyuan City, Taiwan, to analyze chemical pollution levels. Cell-based high-throughput and high-content screening assays were employed to evaluate the toxicity. The results from chemical analyses and toxicity tests were combined using the Toxicological Priority Index (ToxPi) to rank the toxicity of water samples. The results showed that most river water samples, tested at four dilution concentrations, did not exhibit significant cytotoxicity. However, androgen receptor activity showed seasonal variations, and estradiol production in the steroidogenesis pathway was affected, indicating potential endocrine-disrupting activity with seasonal fluctuations. In addition, no significant effects were observed on mitochondrial function or in the micronucleus test, suggesting normal liver cell function and no genotoxicity. We suggest that the indication of endocrine-disrupting toxicity in the water samples points to the presence of potential endocrine disruptors, making it an important direction for future water sample toxicity assessments. By integrating alternative testing and computational toxicology, this study provides a more comprehensive understanding of the hazards posed by water quality, helping to ensure environmental and public health.

1.06.P-Th018 Impact of Indoor VOC Exposure on Atopic Dermatitis and Rhinitis: Insights from the Korean National Health and Nutrition Survey

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This study aimed to explore the association between indoor volatile organic compounds (VOCs) and the prevalence of atopic dermatitis and rhinitis. Using data from the 8th Korean National Health and Nutrition Examination Survey (KNHANES) conducted from July 2020 to August 2021, we analyzed 1,540 adults aged 19 and older who completed a survey regarding the diagnosis of atopic dermatitis. Exposure to VOCs, such as benzene (N-Acetyl-S-(phenyl)-L-cysteine), toluene (N-Acetyl-S-(benzyl)-L-cysteine), ethylbenzene, styrene, xylene, acrolein, 1-bromopropane, and 1,3-butadiene, was assessed using urinary biomarkers analyzed through LC-MS/MS. Statistical analysis revealed that participants with atopic dermatitis and rhinitis had significantly higher geometric mean (GM) concentrations of benzene. Specifically, the GM (SD) concentration of benzene was higher in individuals with atopic dermatitis (5.642 (1.124)) compared to those without (4.858 (1.033)), and in those with rhinitis (5.042 (1.067)) compared to those without (4.864 (1.037)). Additionally, the odds ratio for atopic dermatitis associated with benzene exposure was elevated (OR = 2.156, 95% CI: 1.526-3.048), while the association with rhinitis was weaker and not statistically significant (OR = 1.182, 95% CI: 0.953-1.466). The variability in VOC exposure was also influenced by household ventilation; households with proper ventilation had lower benzene concentrations (GM = 4.865 (1.033)) compared to households without ventilation (GM = 7.716 (1.310)). These findings suggest that specific VOCs, including benzene, may exacerbate atopic

conditions, highlighting the importance of improved indoor air quality and ventilation in mitigating exposure risks.

1.06.P-Th019 Digital Product Passports for Improved Consumer Transparency and Health Impact Awareness

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The growing demand for transparency in product sustainability and safety has sparked the development of Digital Product Passports (DPPs), offering significant potential for improving consumer awareness of health risks and environmental impacts throughout a product's lifecycle. DPPs decentralized and comprehensive product information, providing detailed data on chemical compositions, material sourcing, and health risks, including the identification of hazardous substances. These passports are poised to support informed, health-conscious, and sustainable consumer choices, but the effective implementation of such systems remains a challenge. This work aims to address two key goals: (i) identify the primary challenges in creating accessible, standardized, and informative DPPs, and propose practical solutions to overcome these hurdles, and (ii) explore how DPPs can enhance consumer transparency and raise awareness of health impacts. A multi-faceted approach is presented, focusing on several strategies to ensure DPPs are both accessible and informative: Standardized Formats: Establishing consistent data formats for critical health and safety information, making it easier for consumers to evaluate product safety and environmental impacts. Leveraging data standards like the FAIR (Findable, Accessible, Interoperable, Reusable) principles will ensure DPPs are universally compatible and understandable. Improved Consumer Accessibility: Utilizing mobile technology such as QR codes or NFC chips enables consumers to access DPPs instantly via their smartphones. This approach increases product data accessibility in real-time, empowering consumers with essential health and sustainability information at the point of use. Health Impact Information Integration: By incorporating health-related data from internationally recognized toxicity databases (e.g., REACH in the EU), DPPs can provide consumers with up-to-date information about chemical risks, allergens, and other harmful substances. This ensures that product labels are not only comprehensive but also reliable, helping consumers make health-conscious decisions. Through the adoption of standardized data formats, enhanced accessibility and integration of health impact information, DPPs can provide health impact awareness by helping consumers understand exposure risks associated with certain materials and guiding safer use of them.

1.07.A Advancing the Environmental Safety Assessment of Chemicals through New Approach Methodologies (NAMs): From Early Development to Practical Applications

1.07.A.T-01 A High-Throughput Alternative Approach for Acute Fish Toxicity

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At present there are only two New Approach Methods (NAMs) recognized for acute fish toxicity. OECD TG429 uses the rainbow trout gill cell line RTgill-W1 in combination with plate-reader based viability endpoints to predict acute fish toxicity. The method works for a wide range of chemicals, but is limited in throughput due to the large well-format (24-well) and the requirement for analytical verification of the exposure concentration. We aimed to develop a high-throughput alternative in 384-well format, using TG429 as a starting point. In addition to the plate-reader based viability assay, two additional assays were implemented: (1) an image-based cell viability assay (using Hoechst and propidium iodide); and (2) high-throughput phenotypic profiling (with Cell Painting) to detect sub-cytotoxic effects. We then tested 225 chemicals, each at eight concentrations and in four independent experiments. We derived effective concentrations (typically EC50) for the viability assays and phenotype altering concentrations (PAC) for the Cell Painting assay. These potency values were compared with published results from RTgill-W1. To compare with in vivo data from rainbow trout and fathead minnow, the nominal potency values were converted to predicted exposure concentration with a new in vitro disposition model. Of the 225 tested chemicals, 151 (67%) were active in the Cell Painting assay, some of which were also active in one or both of the cell viability assays. A total of 46 chemicals have been tested in RTgill-W1 in previous studies. For all but two chemicals, the PAC was lower than the EC50 estimates of previous studies, indicating that the PAC can serve as a lower bound to the EC50 for cell viability. In vivo lethal concentrations (LC) were collected for 93 chemicals. For 59 chemicals the adjusted in vitro effect concentration could be compared to the in vivo LC50. A root-mean-squared error of 1.27 (in log10 μM) was obtained; for 73% of chemicals the in vitro potency was protective (i.e., comparable or lower than the in vivo potency). This result is equivalent to nominal results obtained from TG429, with TG429 reaching a root-mean-squared error of

1.14 when in vitro disposition is measured experimentally. To conclude, TG429 gives slightly more accurate results, but our approach enables the high-throughput testing of chemicals, which can assist in prioritization of chemicals in environmental safety testing. This abstract does not reflect USEPA policy.

1.07.A.T-02 Critical Membrane Concentration and Baseline Toxicity of Organic Pollutants in a High-Throughput Rainbow Trout Cell Assay Used for Environmental Hazard Assessment

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The traditional fish acute toxicity test commonly used in environmental risk assessment requires a large number of fish and uses mortality as the primary endpoint, which raises ethical concerns and poses logistical challenges. To address these issues, we developed a high-throughput, image-based in vitro assay using a cell line from the rainbow trout, RTgill-W1. This assay aligns with New Approach Methodologies (NAMs) and the aim of modernizing hazard assessment by providing a reliable, efficient, and animal-free method for evaluating the toxicity of environmental pollutants. The RTgill-W1 assay was optimized for a 384-well format, enabling automated, high-throughput testing with the use of live-dead staining via Hoechst 33342 and propidium iodide, which allows accurate assessment of cell viability in response to toxic exposures. It was applied to evaluate the cytotoxicity of 42 neutral and ionizable organic chemicals, spanning five orders of magnitude in hydrophobicity. Freely dissolved effect concentrations of the tested chemicals were measured using solid-phase microextraction to determine the critical membrane concentration, and a mass balance model was applied to predict the nominal baseline toxicity. As baseline toxicity represents the minimum toxicity elicited by chemicals through the disruption of cellular membrane function, quantitative structure-activity relationships (QSARs) for baseline toxicity can serve as an anchor for effect assessment. These models can provide valuable insights into potential cytotoxic mechanisms, guide appropriate dosing, and identify issues related to experimental performance or assay quality. The majority of the tested chemicals were found to be baseline toxicants and 17 chemicals showed higher toxicity than predicted baseline toxicity. The RTgill-W1 image-based assay is robust, simple, and suited for high-throughput screening. Its reliance on live-dead staining rather than metabolic activity determination minimizes assay artifacts and ensures reproducibility, providing an ethical, reliable, and effective alternative to conventional fish toxicity tests. This assay not only improves sustainability in environmental toxicology, but also contributes to a better understanding of chemical hazards in the aquatic environment. It provides a scalable solution that aligns with regulatory goals for modern, animal-free toxicity testing and improved environmental risk assessment.

1.07.A.T-03 Grouping and Assessment of Chemicals for Hazard and Risk Assessment By High Content Analysis Using The Zebrafish Embryo as an Alternative Model

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For ethical reasons and to keep pace with the development of new compounds, there is a growing demand for using new approach methods (NAMs) in hazard and risk assessment. The zebrafish embryo (*Danio rerio*) (ZFE) has been extensively used as an alternative model for hazard characterization and to explore the mechanistic basis of chemical toxicity. Its primary advantage lies in its ability to represent the complexity of an entire organism while offering diverse endpoints that are able to capture various modes of action of chemicals. Traditionally, ZFE-based assessments rely on microscopic observations, which are prone to observer bias and have limited capacity for deriving concentration-response analyses for specific endpoints. Within the EU project PrecisionTox, we utilized the ZFE model alongside other alternative organismal models to characterize 200 chemicals spanning diverse modes of action and use categories. Our approach involved automated, unbiased phenotype assessments, cardiovascular and behavioral effects. Morphological phenotypes were evaluated using automated positioning and imaging of embryos. Videos were captured to analyze heart rate and cardiovascular effects. Behavioral endpoints such as spontaneous tail contractions, photomotor responses, and locomotor responses were systematically assessed using video analysis. From over 50 endpoints, concentration-response curves and corresponding EC50 values were derived. To estimate the specificity of observed effects, we compared the observed effect concentrations with the predicted ZFE baseline toxicity. For pharmaceuticals and pesticides with known specific modes of action, high sensitivity ratios (predicted baseline toxicity LC50 / observed EC50) confirmed that our approach could detect specific effects. In a case study involving acrylamides and imidazoles, we compared chemical structure-based grouping with observed effect patterns. While

significant overlap was observed between the two, deviations highlighted the value of combining both approaches for read-across scenarios in risk assessment. By analyzing the effect patterns of all 200 test compounds and comparing them with known modes of action, we are further evaluating the diagnostic potential of our automated assessment methodology. This work received funding by the European Union's Horizon 2020 research and innovation program project Toward Precision Toxicology: New Approach Methodologies for Chemical Safety (PrecisionTox), grant agreement no. 965406. We gratefully acknowledge access to the platform CITEPro (Chemicals in the Environment Profiler) funded by the Helmholtz Association for ZFE experiments and pKa determination.

1.07.A.T-04 Sensitivity of Fishes to Polycyclic Aromatic Hydrocarbons

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental chemicals that induce toxicity in fish by activating the aryl hydrocarbon receptor (AhR). Fishes are known to differ dramatically in sensitivity to AhR activation by the prototypical AhR agonist TCDD and PAHs. Predictive models linking AhR activation by TCDD to early life-stage mortality have been developed to aid in risk assessment, however this model is not applicable to PAHs. This research aims to 1) develop a predictive model linking AhR activation and early life-stage mortality for PAHs and 2) explore molecular mechanisms underlying differences in species sensitivity. This research utilized a standardized in vitro reporter gene assay to develop species sensitivity distributions based on half maximal effect concentrations (EC50s) for transactivation of the AhR by benz[a]anthracene and 8-methylbenz[a]anthracene in 18 species of fish. To determine whether differences in sensitivity to AhR activation correspond with differences in sensitivity to early life-stage mortality embryos of fathead minnow (*Pimephales promelas*), zebrafish (*Danio rerio*), and brook trout (*Salvelinus fontinalis*) were exposed to PAHs by microinjecting a standardized volume of chemical solution directly into the yolk sac of embryos. Lastly, in silico docking studies and primary sequence analyses were performed to determine binding potential energies and investigate the role of receptor subdomains in differences in species sensitivity. Interspecies variation in sensitivity to AhR activation by PAHs exceeded 500-fold and there is a strong and significant relationship between sensitivity to AhR activation (EC50) and sensitivity to early life-stage mortality (LD50; $p = 0.007$, $R^2 = 0.86$). Predicted LD50s made using this approach averaged an accuracy of 3.3-fold relative to measured LD50s. While no significant correlation exists between binding potential energies and AhR activation ($p = 0.11$, $R^2 = 0.36$), there was a significant correlation between the size of the glutamine-rich and acidic-rich subdomains of the transactivation domain of the AhR ($p = 0.0005$, $R^2 = 0.65$). Due to the abundance of PAHs in the environment, differences in sensitivity across species, and practical limitations of performing traditional toxicity tests across numerous species, developing in vitro and in silico tools to assess these toxicities more efficiently could be essential for more objective ecological risk assessment of these chemicals.

1.07.A.T-05 In Silico Prioritization of Flame Retardants: Integrating Molecular Docking and Dynamics Simulations to Assess Endocrine Disruptor Potential

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Numerous flame retardants (FRs) have been reclassified due to their recognized hazards, including potential endocrine-disrupting properties. This study systematically screened potential endocrine-disrupting chemicals (EDCs) among commonly used FRs in the EU through an advanced in silico-based workflow integrating molecular docking and molecular dynamics (MD) simulations. Molecular docking was conducted to evaluate the binding affinities of FRs to Androgen Receptor (AR) and Estrogen Receptor alpha (ER α), prioritizing compounds with strong interactions for further analysis. Detailed 100 ns MD simulations assessed the dynamic behavior, binding stability, and interaction resilience of the selected FR-receptor complexes. Key parameters, including Gibbs free energy (ΔG), root-mean-square deviation (RMSD), and hydrogen bond persistence, were normalized and compared to reference compounds to evaluate EDC potential. Among the FRs analyzed, N,N'-ethylenebis(3,4,5,6-tetrabromophthalimide) exhibited strong binding affinity and dynamic EDC-like behavior with both receptors, suggesting significant endocrine-disrupting potential. Four FRs, including 6H-dibenz[c,e][1,2]oxaphosphorin 6-oxide and N,N'-ethylenebis(3,4,5,6-tetrabromophthalimide), were

identified with strong receptor interactions and notable regulatory data gaps. Additionally, Phenol, isopropylated, phosphate (3:1) showed unstable interactions with ER α despite being listed in EU EDC assessments, while high-production compounds such as Cyanuric acid remain absent from regulatory lists, emphasizing the need for further evaluation. By integrating molecular docking and robust MD simulations, this study provided comprehensive insights into FR-receptor interaction dynamics and structure-activity relationships. The findings highlight the potential of in silico methods to support regulatory frameworks by identifying high-risk FRs and addressing existing regulatory gaps, facilitating enhanced chemical safety assessments. This work was supported by Korea Environment Industry & Technology Institute (KEITI) through Technology Development Project for Safety Management of Household Chemical Products, funded by Korea Ministry of Environment (MOE)(RS-2023-00215309)

1.07.B Advancing the Environmental Safety Assessment of Chemicals through New Approach Methodologies (NAMs): From Early Development to Practical Applications

1.07.B.T-01 Assessing a Primary Hepatocyte Monolayer Culture System for Studying Pharmaceutical Clearance in Fish

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The presence of active pharmaceutical ingredients (APIs) and their metabolites in aquatic ecosystems presents potential risks to non-target organisms such as fish. Fish are extensively used in the environmental risk assessment (ERA) of human pharmaceuticals, yet our understanding of their drug metabolism and transport capabilities is limited. While in vivo tests are currently being used to help fill this knowledge gap, they present major ethical concerns and uncertainties in terms of organism-specific pharmacokinetics. In vitro cell-based models can provide valuable information to help support prioritisation strategies for in vivo studies, and, in addition, help validate existing in silico models. To this end, we assessed the use of a rainbow trout (*Oncorhynchus mykiss*) primary hepatocyte monolayer culture system for predicting hepatic clearance for five diverse APIs, and for building further understanding on how these APIs affect drug metabolism and transport systems in fish. Briefly, this involved the isolation of primary hepatocytes from the livers of rainbow trout, culturing the liver cells as monolayers under physiologically relevant conditions and then exposing them to different concentrations of the selected APIs for up to 24h. Samples of culture medium and cells were subsequently collected to analyse parent API depletion (via LC-MS/MS), metabolic enzyme activity (ethoxyresorufin-O-deethylase, EROD) and the relative expression of metabolism- and transport-related target genes. The monolayers were able to clear all tested APIs (0.1 and 1 μ M nominal exposure concentrations) to varying extents (26-100%). Generally, clearance rates were consistent across replicate cultures for each of the studied APIs, and, in most cases, extrapolated in vivo clearance rates were comparable to those observed in humans. EROD activity was detectable and inducible in the hepatocyte monolayers, with three APIs (at 100 μ M) causing significant EROD induction after 8h. Two of the APIs tested (at 0.1-1 μ M) caused significant upregulation of key genes involved in API metabolism and transport after only 2h. Taken together, our findings show that rainbow trout primary hepatocyte monolayers provide a relevant and replicable in vitro model for predicting the clearance of diverse APIs and for understanding the effects of API exposure on metabolism and transport systems in fish, hence providing valuable data for validating in silico models and informing in vivo studies. This work was funded by Servier Laboratories as part of PREMIER (Prioritisation and Risk Evaluation of Medicines in the Environment). PREMIER has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 875508. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and the European Federation of Pharmaceutical Industries and Associations.

1.07.B.T-02 Refining the Environmental Risk Analysis of Pharmaceuticals to Fish By Considering Fish (Versus Human) Blood Plasma Binding in the Fish Plasma Model

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In the project PREMIER (Prioritization and Risk Evaluation of Medicines in the Environment) a fish decision tree has been developed to minimise in vivo fish testing for legacy human pharmaceuticals. Part of this decision tree relies on the Fish Plasma Model (FPM), which uses the lipophilicity (LogD) of an active pharmaceutical ingredient (API) to predict its concentration in the blood plasma of exposed fish from the measured API concentration in the surrounding water. If the predicted environmental concentration (PEC) is below the therapeutic water concentration (TWC) (Eq.1), the fish plasma

concentration will be below the human therapeutic concentration (HtPC) and fish are unlikely to experience pharmacological effects. A key assumption in the FPM is that the unbound fraction of API (i.e. not bound to plasma proteins) in blood plasma, which is available for pharmacological action, is the same in fish as it is in humans. We test this assumption, building on previous published work. Circa 40 APIs, including basic/cationic, acidic/anionic and neutral compounds with wide ranging physico-chemical and pharmacokinetic properties, were subjected to plasma protein binding assays using blood plasma sampled from rainbow trout (pH 7.8-7.9, 11-15°C), fathead minnows or Koi carp (pH 7.7, 25°C) or humans (pH 7.4, 37°C). Assays were performed by equilibrium dialysis with pH matched buffers; only unbound API was able to traverse the 8 kDa dialysis membrane from the plasma to the buffer, while protein-bound API remained in plasma samples. The fraction unbound (f_u) for acidic/anionic APIs was generally higher for fish relative to humans. Most notably f_u rainbow trout / f_u humans (Rfu) was 43 and 35 for Ibuprofen and Mycophenolic acid (MPA), respectively. Conversely Rfu was ~0.1 for some basic/cationic APIs. Collectively these data evidence the need to refine the FPM. Therapeutic water concentrations (TWCs) predicted for APIs using the refined FPM, incorporating Rfu_{human}, were lower for acidic/anionic APIs (up to 43 times) than those predicted without Rfu, while TWCs were up to 10 times higher for some basic/cationic APIs. To help explain these differences, inter-species differences in plasma proteins and their capabilities for binding APIs are being explored alongside API molecular properties. Incorporating these refined predictions of TWC in the fish decision tree would indicate that fish testing may be justified for more anionic APIs than originally identified. This contribution has been prepared within PREMIER, a project that has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 875508. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA. The opinions expressed in this study are those of the authors only and do not necessarily reflect the opinion of the institutions to which the authors are affiliated or the opinion of all PREMIER partners.

1.07.B.T-03 In Vitro Evaluation of Pharmacokinetics in Fish Exposed to Complex Pharmaceutical Mixtures

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Animal testing of pharmaceuticals for the safety to the environment follow international standards aimed at assessing the impact of xenobiotics on non-target organisms, particularly in aquatic environments where significant concentrations of pharmaceuticals, especially in fish, are prevalent [1, 2]. Most of the active substances are present in the environment in the form of mixtures. It is rare to detect only one active substance in an environmental sample. Our understanding of the effects of pollutants present in these mixtures is limited compared to that of individual xenobiotics [3,4]. Pharmacokinetic (PK) studies serve as another essential approach for evaluating the risks associated with exposure to potentially harmful chemicals and have long been used in human risk assessment. These studies investigate the fate of chemicals in the body, including absorption, distribution, metabolism, and excretion (ADME), as well as the potential for accumulation and the resulting effects on human health. The adaptations of techniques used in human studies have enabled the application of PK studies in fish [5]. In vivo PK studies have been instrumental in determining the distribution of active pharmaceutical ingredients (APIs) and assessing potential contaminant levels within fish [6, 7, 8]. Another adaptation of PK studies in fish is the development of in vitro fish cell cultures that can be used to evaluate ADME, thereby aiming our understanding of the toxic effects of chemicals and the potential risks to fish health and the environment. This study aims to deploy fish cell culture systems for high-throughput analysis of PK of environmentally-occurring pharmaceuticals. It seeks to guide the development of effective risk management strategies, to protect fish populations and ensure the health of aquatic ecosystems. AV was in receipt of a scholarship (awarded to CJH and LPB). The authors would like to thank the staff in the Biological Facility Unit at the Franklin Wilkins Building, King's College London and Sparsholt College for the care and supply of experimental animals. This work was funded by a Biotechnology and Biological Sciences Research Council (BBSRC) iCASE studentship with AstraZeneca under a BBSRC Collaborative Training Partnership and support from King's College London and Imperial College London.

1.07.B.T-04 Critical review of in vitro dosing methods for hydrocarbon UVCBs

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Alternative approaches to traditional animal testing are being promoted to support regulatory chemical assessments. These approaches include in vitro test methods and can be used in multiple contexts, from prioritization and screening to supporting chemical grouping and read-across. It is anticipated that in vitro tests will eventually replace whole organism in vivo testing. An important challenge of most in vitro testing methods is establishing, maintaining, and confirming defined exposure concentrations throughout typical test durations. This is particularly challenging for petroleum UVCBs (substances of Unknown, Variable composition, Complex reaction products, or Biological origin) that typically contain a large number and variety of hydrophobic and (semi)volatile hydrocarbon constituents (HC) that are prone to evaporative and sorptive losses. The ability to deliver and maintain stable petroleum substance (PS) exposures in in vitro test systems is challenged by several factors, including: the high surface area to volume ratios of most multi-well plates, which increases the likelihood of sorption to plate walls; the inability to seal some test vessels (e.g. volatile constituents can escape open test vessels and may contaminate neighboring plate wells); poor solubility of hydrophobic constituents in biological media; compatibility with small testing volumes; and presence of lipids and proteins in biological media which may differentially bind individual constituents. Here we present the findings from a critical review describing the challenges of dosing UVCBs into conventional in vitro test setups and identifies relevant assays, dosing methods, and potential adaptations that may improve in vitro testing of UVCBs and other difficult-to-test substances. Approaches best suited for regulatory testing of PS and areas of future research are also identified. This work was funded by CONCAWE.

1.07.B.T-05 Predicting the Sensitivity of Reptiles to Dioxin-Like Chemicals: A Quantitative Adverse Outcome Pathway Approach

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Reptiles represent the least-studied group of vertebrates in ecotoxicology. This lack of toxicity data represents a significant uncertainty in ecological risk assessments of this taxon. However, the process for assessing the toxicity of chemicals is undergoing a fundamental shift from an emphasis on whole-animal testing of apical-level toxicities to a greater focus on conserved mechanistic end points. A prior study developed a cross-species quantitative adverse outcome pathway (qAOP) model capable of predicting full dose-response for early life stage mortality following exposure to dioxin-like chemicals (DLCs) in any species of fish or bird using the 50% effective concentration (EC50). Calculated EC50s were derived from a standardized in-vitro aryl hydrocarbon receptor (AHR) transactivation assay of COS-7 cells transfected with an AHR cloned from the species of interest. The objective of this study was to evaluate if this same qAOP could accurately predict toxicities to reptiles. Specifically, the AHR1 and AHR2 isoforms were cloned from the common snapping turtle (*Chelydra serpentina*) as a model reptile. The four DLCs, namely 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), 2,3,7,8-tetrachlorodibenzofuran (TCDF), and 3,3',4,4',5-pentachlorodiphenyl (PCB 126), were assessed in the standardized in-vitro AHR transactivation assay. These chemicals are in alignment with a previous study investigating early-life toxicity for snapping turtles in-vivo. Using the qAOP, the fold difference between the model and the experimental data with the snapping turtle was determined to be between 10 and 15-fold across the investigated chemicals. This currently falls roughly within the one order of magnitude acceptability when performing risk assessments, and the larger fold-difference is likely due to inaccuracies during the in-vivo exposures, which is expected given the logistical constraints involved with the exposure method. However, this study demonstrates the successful utility of predictive toxicology in assessing the sensitivity of difficult to test species, such as reptiles, to environmental pollutants of concern. This serves as a proof of concept and suggests that relationships observed in other predictive models may be representative of reptiles as well, ultimately contributing to an increase in available toxicity data without the traditional largescale lethal sampling associated with in-vivo assays.

1.07.C Advancing the Environmental Safety Assessment of Chemicals through New Approach Methodologies (NAMs): From Early Development to Practical Applications

1.07.C.T-01 Advancing Ecological Risk Assessment: Integrating High-Throughput Data for Pesticide Evaluation

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Pesticide ecological risk assessment relies heavily on vertebrate testing, which is time-consuming, costly, and ethically challenging. New approach methodologies (NAMs), including high-throughput assays (HTAs) like U.S. EPA's ToxCast, offer faster, more animal friendly alternatives. This study evaluates the ability of HTAs to approximate risk quotients (RQs) derived from traditional tests for 120 pesticides across chemical classes and modes of action. While most HTAs underestimated risks, certain assays, particularly cytochrome P450 (CYP) enzyme assays, performed well for herbicides and non-target taxa. Acute risks were better predicted than chronic, and nonpolar narcosis chemicals showed strong alignment. However, gaps remain in assay coverage for target-specific risks, especially neurotoxic insecticides. HTAs show promise as supplemental tools but require further development to address critical gaps and improve accuracy, supporting their integration into sustainable pesticide risk assessment frameworks.

1.07.C.T-02 Clearing the Waters: How Daphnids Chart a New Course for Vertebrate-Free Aquatic Toxicity Testing

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Environmental impact assessments of chemicals involve aquatic toxicity tests using algae, daphnids, and fish, targeting different trophic levels. Efforts to reduce vertebrate animal testing necessitate alternatives, such as RTgill-W1 cell line and zebrafish embryo assays. Yet they may not adequately cover neurotoxic modes of action or biotransformation capacities. Moreover, it needs to be cleared if amphibian-specific acute toxicity could be of concern, although few such data are available. We hypothesized that daphnids exhibit greater sensitivity than fish and amphibians in acute toxicity tests, especially for neurotoxic compounds and for allyl-alcohol as the currently unique example of relatively low sensitivity due to different biotransformation in alternative models compared to juvenile vertebrates. If not disproven, our findings could overcome the current limitations of alternative methods for acute aquatic toxicity. Using the EnviroTox Database, we analyzed acute toxicity data, selecting compounds in line with OECD Test Guidelines (TGs) 202 (daphnids) and 203 (fish) and excluding values exceeding water solubility limits. Compounds were categorized based on neurotoxic mode of action (MoAs) from ASTER, TEST, IRAC, and other recent MoA annotations. Median daphnid EC50 and fish LC50 ratios (Rdf) were calculated, yielding about 140 neurotoxic compounds for acute daphnid and fish data and a total of 805 chemicals. We also extracted 93 amphibian EC50 values and calculated daphnid-to-amphibian ratios (Rda) and we analyzed outliers of low sensitivity in the RTgill-W1 and zebrafish embryo assays. Biological variability is characterized by coefficients of variation between 50%-100% for both daphnids and fish. Using median Rdf values, daphnids were protective for 96% of 805 compounds, independent of mode of action. For neurotoxic compounds, only a few chemicals no longer on the market exceeded Rdf >10, identifiable by structural alerts. Allyl-alcohol showed an Rdf below 1 and similar trends were seen in Rda values.

Daphnid data covered low sensitivity outliers for the RTgill-W1 and zebrafish embryo tests effectively. Our research supports replacing juvenile fish toxicity tests, aligning with OECD standards, and broadens environmental protection. This shift may eliminate further vertebrate testing in environmental toxicology. We plan to extend the analysis to chronic aquatic toxicity data. The authors declare not to have any conflict of interest. This work was funded by the Swiss data science center (SDSC) grant "Enhancing Toxicological Testing through Machine Learning" (project No C20-04), partly carried out in the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC) and has received funding from the European Union's Horizon Europe research and innovation program under Grant Agreement No 101057014. The work of MP at the Medical University is co-financed via PARC and the Austrian Federal Ministry for Climate Action, Environment, Energy, Mobility, Innovation and Technology, Department V/5 Chemicals Policy and Biocides.

1.07.C.T-03 Fish and Amphibian Eleutheroembryo Assays as New Approach Methodologies for Regulatory Assessment of Endocrine Activity of Chemicals – A European Industry Perspective

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Regulatory assessment of endocrine-disrupting (ED) properties of chemicals relies upon extensive use of

animals. For aquatic vertebrates, the recommended fish and amphibian tests require hundreds of animals per substance to screen for endocrine activity, and nearly two thousand to investigate adverse effects. Recently, OECD-validated test guidelines (TGs) for eleutheroembryo assays have been introduced as New Approach Methodologies for the assessment of endocrine activity of chemicals in aquatic vertebrates. Because they do not feed independently, eleutheroembryos are not considered as vertebrate test animals. Under certain conditions, defined in the ECHA-EFSA guidance for the identification of ED properties of pesticide and biocide active substances, the *Xenopus* Eleutheroembryonic Thyroid Assay (XETA, OECD TG 248) can be used to assess the activity of substances on select thyroid pathways. Other eleutheroembryo assays using fish species have also been validated by the OECD for the identification of chemicals acting on the estrogen and androgen axes (OECD TGs 250, 251 and 252). The Rapid Androgen Disruption Activity Reporter assay (OECD TG 251) and the Rapid Estrogen ACTivity Test In Vivo assay (OECD TG 252) provide insights on potential effects on downstream steps of the steroidogenesis as well. The conditions for using fish eleutheroembryo assays as alternatives to chronic fish in vivo tests for regulatory assessment of endocrine activity still need to be defined. However, the use of the XETA as an alternative to the Amphibian Metamorphosis Assay (OECD TG 231) in the context of (re)approval of pesticide active substances in Europe effectively resulted in ca. 60% reduction of animal use. Further animal reduction might be expected from the validation of thyroid-specific endpoints in fish TGs required for regulatory evaluation of pesticide and biocide active substances. Though, the replacement of animal tests with eleutheroembryo assays ultimately relies on a thorough understanding of their applicability domains and acceptance by regulatory authorities, which varies amongst countries and jurisdictions. Discrepancies also exist amongst OECD eleutheroembryo TGs regarding e.g., validity criteria, decision logic or statistical analyses, which may complicate the practical implementation of these approaches. This, coupled with lessons learned from the use of eleutheroembryo assays over the last few years call for harmonization of the corresponding OECD TGs.

1.07.C.T-04 Ensuring the Credibility of New Approach Methodologies in Regulatory Toxicology: The Vital Role of Validation and Ring Trials

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The validation of New Approach Methodologies (NAMs) is a cornerstone for their acceptance and integration into regulatory toxicology. This talk delineates the critical aspects of relevance and reliability in the validation process, highlighting the indispensable role of ring trials. Relevance pertains to the extent to which NAMs accurately measure or predict the biological effect of interest, ensuring that the data generated are pertinent to hazard and risk assessment. Reliability, on the other hand, involves the consistency of NAMs performance across different laboratories and conditions, which is crucial for their widespread acceptance. Ring trials, also known as inter-laboratory studies, are emphasized as a fundamental component of the validation process. These trials involve multiple laboratories conducting the same experiments independently to verify the reproducibility and robustness of the NAMs. The insights gained from ring trials help in refining the methodologies and establishing standardized protocols, thereby enhancing the overall confidence in NAMs. The transition from traditional animal-based testing to NAMs represents a significant shift in regulatory toxicology. This transition is driven by ethical considerations, advancements in science and technology, and the need for more human-relevant data. However, the success of this transition hinges on the rigorous validation of NAMs to ensure that they can reliably predict human health outcomes. The integration of NAMs into regulatory frameworks requires a concerted effort to standardize validation processes, implement ring trials, and adhere to guidelines such as Good In Vitro Method Practices (GIVIMP). In conclusion, the validation of NAMs is a multifaceted process that requires careful consideration of relevance and reliability. Ring trials play a pivotal role in this process, providing the necessary evidence to support the robustness and reproducibility of NAMs. Adhering to guidelines such as GIVIMP ensures that NAMs are developed and validated to the highest standards, facilitating their acceptance and integration into regulatory toxicology. The insights from key publications highlight the ongoing efforts to refine validation processes and underscore the importance of rigorous and standardized approaches to ensure the reliability and relevance of NAMs.

1.07.C.T-05 Solutions For Regulatory Uptake of NAMs - Perspective From The Science Policy Interface

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Scientists worldwide have contributed to considerable progress in developing new approach methodologies (NAMs) for the science driven paradigm change in regulatory safety assessments of chemicals. These methods have been welcomed not least because of a desire, reflected in the wider ethical

views of society, to curtail the use of animals for toxicity testing but also for their potential to make regulatory safety assessments more efficient and cost-effective. The incorporation of NAMs into the regulatory processes globally has been inhibited by technical uncertainty (including scientific methods and regulation) but there are also societal considerations which affect the appetite for NAMs. These barriers and their inter-relationship have become a subject of research themselves. In the presented study empirical research determined the sociotechnical barriers perceived by key stakeholders and possible solutions defined with the stakeholder input in a follow-up study. Barriers to the uptake of NAMs are characterized by high complexity and the need for an increased focus on societal aspects. Key barriers identified include i) Numerous vicious circles work against the uptake of NAMs ii) Remaining lack of consensus on scientific readiness iii) The need to reconsider the governance framework that underpins NAMs and iv) Lack of familiarity, trust, and confidence. Where there is broad consensus on solutions to overcome the identified barriers, this study was successful in outlining concrete measures for the areas of i) regulatory use of data ii) education and training strategies and iii) routes towards validation. With regards to the remaining areas of moderate and indeterminate consensus, further enquiry to refine the questions at issue and determine what actions are needed for greater acceptance of NAMs for regulatory purposes is needed. It is crucial to keep the different perspectives, needs and doubts of different stakeholder groups in mind when moving forward in creating policy options. Identifying existing, and fostering underdeveloped areas of broad consensus is vital to successful policy transition. This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 965406.

1.07.P Advancing the Environmental Safety Assessment of Chemicals through New Approach Methodologies (NAMs): From Early Development to Practical Applications

1.07.P-Mo001 OECD 249 Fish Gill Cell Assay: Evaluating Its Applicability for Industrial Chemical Toxicity Prediction

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This study evaluates the practicability and accuracy of the RTgill-W1 fish cell assay (OECD 249) as a promising in vitro method to predict acute fish toxicity, addressing growing animal welfare concerns associated with standard acute fish toxicity tests (OECD 203) and focusing on industrial chemicals including difficult-to-test substances.

We initially tested two distinct groups of industrial chemicals with varying physicochemical properties. The OECD 249 assay was conducted in two laboratories to assess interlaboratory reproducibility. Results were compared with in vivo acute fish toxicity data and in silico predictions. Key findings include strong interlaboratory reproducibility, demonstrating the robustness of the method. Correlation between in vitro and in vivo results varied between the two substance groups, with one group showing excellent alignment, while the other displayed weaker correlation. For one group, in vivo results aligned better with in silico predictions than with in vitro tests. The study highlights both advantages and challenges of the OECD 249 assay. Advantages include faster testing time, animal welfare benefits, and suitability for initial assessments of multiple chemicals. Challenges involve pending regulatory acceptance, optimization of plate pre-coating procedures, and limitations in capturing all modes of action relevant to in vivo fish toxicity. Our findings suggest that an integrated approach, considering multiple lines of evidence, could help address variabilities associated with alternative methods. Further exploration of correlation levels between alternative methods and traditional tests may inform their regulatory acceptance. Ongoing work is expanding the chemical test set to include more difficult-to-test substances to characterize assay performance across diverse chemical classes and solubility profiles, aiming to better define the assay's applicability domain, limitations, and optimization opportunities.

With further refinement, the OECD 249 assay could provide a practicable alternative for rapid, cost-effective environmental toxicity estimation of industrial chemicals, potentially reducing reliance on fish acute toxicity testing. This study contributes to the scientific understanding and potential regulatory acceptance of the fish gill assay as part of a comprehensive strategy for aquatic toxicity assessment, aligning with the 3R principles (Replacement, Reduction, Refinement) in toxicology testing.

1.07.P-Mo002 Setting the Bar: Characterizing Variability Across Standard Acute Fish Toxicity Assays

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Regulatory environmental toxicology relies on experimental results from a handful of standardized toxicity tests including the fish acute toxicity assay (e.g., OECD 203, EPA OPPTS 850.1075). Several animal alternative assays have been developed to replace the acute fish toxicity (AFT) assay including the

fish embryo toxicity (OECD 236) test, RTgill cell cytotoxicity assay (OECD 249), as well as computational approaches. These new approaches must be easily transferred to new labs, statistically robust, reproducible, reliable, and accurate. That is, the replacement assay must produce the same result as the AFT (e.g., LC50). Implicit in this comparison is the assumption that the AFT is robust, reliable, and reproducible. The reliability and reproducibility of the AFT are largely unknown, and widely taken for granted during animal alternative assay validation exercises. In this presentation, we summarize the development and curation of an AFT reference database containing LC50 values, 95% confidence intervals, and relevant experimental parameters, as available. The database includes over 700 chemicals, 1782 unique studies, and 5 unique freshwater fish species allowed under standard test guidelines. This database was statistically probed to describe variability and uncertainty in the AFT assay. Intra-laboratory, inter-laboratory, and interspecies variability were explored. The influence on physicochemical properties and MOA on AFT variability were also explored. Machine learning models were developed to characterize AFT variability, informed by experimental variability within a CAS and within the calculated LC50 value (e.g., utilizing the 95% confidence interval). This information can be used to set expectations on AFT assay performance and help contextualize results and expectations for alternative assays.

1.07.P-Mo003 Comprehensive Toxicity Profiling of 26 BPA Alternatives Based on Eight Cell-Based in vitro assays and Abiotic CYP Oxidation Test

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Bisphenol A (BPA, CAS 80-05-7) is an endocrine disruptor linked to numerous negative health outcomes. Many BPA alternatives exist and are marketed, but their environmental and human health hazards are less well-defined than for BPA. High-throughput in vitro bioassays can help fill these gaps with early identification of problematic alternatives. However, no standardized methods exist to interpret and integrate results from multiple bioassays and toxicity domains. This case study proposes a set of four ratios to compare specific effects and cytotoxicity against baseline toxicity for 26 BPA alternatives and BPA across eight bioassays, including phase I metabolic activation. The in vitro test battery covered eight endpoints and cytotoxicity, targeting key toxicity pathways: estrogenicity (ER?), metabolic disruption (PPAR?), xenobiotic metabolism, genotoxicity, oxidative stress, mitochondrial toxicity, and neurotoxicity. Phase I metabolism was simulated using the abiotic CYP (aCYP) catalyst TDCPP (CAS: 91463-17-1). Fifteen bisphenol alternatives showed equal or higher cytotoxicity than BPA. Eighteen bisphenols exhibited specific estrogenic activity, with most specificity ratios highest in compounds structurally most similar to BPA. We also observed PPAR? activation and several compounds showed mitochondrial toxicity. Only trans-TCMD (CAS 2694-23-7) and racemic TCMD (CAS 3010-96-6) exhibited minimal effect concentrations across all assays. After aCYP oxidation minimal decrease of concentrations of the parent compound could be observed for most alternatives. Only Pergafast (CAS 232938-43-1), BTUM (CAS 151882-81-4), BPT (CAS 2664-63-3), and BPF (CAS 620-92-8) showed >25% reduction of parent molecule concentration. Cytotoxicity after activation decreased for BPF and BPT, stayed unchanged for Pergafast, and increased for BTUM. Of the 26 evaluated BPA alternatives, only trans-TCMD, and racemic TCMD have acceptable toxicity profiles, because they had exceptionally low cytotoxicity and did not activate any of the eight specific endpoints below baseline toxicity concentrations. This study illustrates how an in vitro-based approach can improve the selection of non-hazardous chemical alternatives, by evaluating overall cytotoxicity, activity across health-relevant endpoints, and effects of simulated metabolism. This work was carried out in the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC) and has received funding from the European Union's Horizon Europe research and innovation program under Grant Agreement No 101057014. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the Health and Digital Executive Agency. Neither the European Union nor the granting authority can be held responsible.

The in vitro bioassays and chemical analyses were conducted using the high-throughput screening platform CITEPro (Chemicals in the Environment Profiler), funded by the Helmholtz Association with additional support from the states of Saxony and Saxony-Anhalt. We extend our thanks to Jenny Braasch for her assistance with the bioassay measurements. We also want to thank Martin Krauss for his advice on the analytical detection of the bisphenol A alternatives and

1.07.P-Mo004 Towards Fish-Specific New Approach Methodologies (NAMs) for Immunotoxicology: Evaluating the Cytokine Tumour Necrosis Factor Alpha (TNFα) as Reference Immunostimulant for Zebrafish In Vitro Studies

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Growing evidence suggests that many environmental pollutants can induce immunomodulatory and immunotoxic effects across species, including fish. Considering the critical role played by the immune system in the maintenance of health, the European Chemical Agency (ECHA) has recently classified immunotoxicity as a priority regulatory challenge. The effects of chemicals on the immune system of fish are typically characterised using *in vivo* experiments. However, scaling up these investigations for the 100,000+ chemicals currently on the market requires the urgent development of fish-specific new approach methodologies (NAMs). In human immunomodulation and immunotoxicity testing, immunostimulants - such as the pleiotropic cytokine tumour necrosis factor (TNF α) - are commonly used to assess the effects of chemicals on activated immune cells. Here, we hypothesise that TNF α can be considered as the reference immunostimulant for fish *in vitro* immunology studies as it is for human studies. Yet, the effects of TNF α on fish cell lines are largely unexplored, especially in the context of NAMs. Given the lack of fish immune cell lines and the widespread expression of cytokine receptors in non-immune cells, we also hypothesised that ZF4 cells can be used as a proxy to detect the immunomodulatory activity of both endogenous and exogenous substances. To address this knowledge gap, we combined traditional ELISA assays with high-content methodologies to perform an in-depth characterisation of the functional (i.e. interleukin 6 release), morphological, and transcriptomic responses of zebrafish fibroblast-like ZF4 cells to TNF α . Our results indicated that ZF4 cells are highly responsive to TNF α , mimicking the response of human cells. Exposure to 10, 25, 50, and 100 ng TNF α /mL induced a rapid concentration-dependent increase in IL-6 secretion over a 24-hour period, with a peak secretion between 15 and 30 minutes. These results were used to design further studies aimed at characterising short-term (15-30 minutes) and longer-term (24 hours) transcriptomic effects via RNAseq and morphological effects via high-content cell imaging approaches. Our findings suggest that exposure to TNF α at 50 ng/mL can provide a robust method for immunostimulation in non-immune zebrafish cells, supporting its application in fish-specific NAMs for environmental toxicology. This work was funded by a Biotechnology and Biological Sciences Research Council (BBSRC) iCASE studentship co-funded by AstraZeneca under the London Interdisciplinary Doctoral Programme (LIDo) (Ref. 2879914). GH was in receipt of a scholarship (awarded to LM-C) co-funded by AstraZeneca. SFO is an employee and share holder of AstraZeneca, a biopharmaceutical company with an interest in the discovery, development and commercialisation of prescription medicines.

1.07.P-Mo005 Evaluation of an Ontology-Driven *In Silico* Profiler Representing Mechanisms of Action Related to Endocrine Disruption

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The application of *in silico* profiling has, over the course of the past three decades, become established as a means by which the acute ecotoxicological profile of a substance might be characterised. Through assigning putative mechanisms of action, this approach is capable of facilitating compound grouping ahead of read-across or QSAR application, and thus of supporting toxicity prediction and data-gap filling. Recent work has re-examined existing *in silico* profilers, with the intention both of establishing consensus and framing judgments within the context of the Adverse Outcome Pathway (AOP). We have, through this study, sought to extend such methodology towards outcomes relevant in chronic ecotoxicity with a primary focus upon endocrine disruption. To this end, a mechanistic-driven ontology, spanning a broad series of endocrine-related domains, has been created. This comprises systems and networks most notably oestrogenic, androgenic, thyroid and ecdysteroid relevant within a wide variety species (mammalian, arthropod, fish, etc.). Each mechanism is defined in terms of its relationship to AOP-supported molecular initiating events (MIEs), amongst which are included direct hormone receptor interactions, inhibition components key within hormone synthesis, and the impairment of hormone distribution. In order to develop structural alerts associated with each of these MIEs, data relating to the activities of substances at the appropriate target sites were retrieved from sources which, alongside the general literature, included ToxCast, ChEMBL and SciBite. Through adoption of techniques such as molecular scaffold analysis, series of defining structural fragments were identified. These were subsequently coded as SMARTS-based rules, ahead of their compilation as a profiling tool the purpose of which, as before, is to facilitate the flagging of compounds which may hold potential to serve as candidates for further toxicological evaluation (be it *in silico* or experimental). So that the coverage of the profiler might be examined, a modified form of the EU PARC endocrine disruptor inventory was screened against it. Through this exercise, several substance classes, predominantly pesticide forms, were noted as lying beyond its present

domains. The reasoning associated with this observation is discussed. This profiler will be made freely available and the intention is that it will ultimately will be incorporated within the MechoA+ scheme.

1.07.P-Mo006 From Data to Decision: Utilizing NAMs for Aquatic Toxicity Assessment and Regulatory Compliance

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The Life Science business of Merck is at the forefront of advancing In Silico New Approach Methodologies (NAMs) for the human and environmental safety assessment of chemicals. With a global portfolio exceeding 300,000 products, we provide high-quality chemicals and tools essential for research and development in both academia and industry. NAMs encompass various technologies, including in silico, in chemico, in vitro, and ex vivo studies, which deliver critical information on chemical hazard and risk assessment without relying on animal testing. As regulatory agencies worldwide increasingly embrace NAMs, their application in regulatory decision-making is becoming a reality, supported by strategic roadmaps and partnerships such as the US EPA New Approach Methods Work Plan, ICCVAM, NICEATM, EU ECHA (REACH, CLP), APCRA, PARC, and EPAA. In this study, we present innovative workflows that integrate in silico NAMs to facilitate global regulatory submissions in compliance with frameworks like the United States Toxic Substances Control Act (TSCA), EU REACH, and REACH-like regulations. These workflows rely on a combination of automated processes and expert evaluations. Our focus on a comprehensive evaluation of aquatic toxicity results obtained by in silico NAMs, highlighting the complexities involved in generating valid, adequate and relevant data for regulatory compliance. Through various use cases, we present the streamlined application of combined (Q)SAR predictions, read-across methodologies, and weight-of-evidence (WoE) approaches while addressing gaps and existing challenges in applying such approaches for aquatic toxicity within the regulatory landscape, particularly for data-poor substances. We aim to illustrate how these methodologies can inform and shape future regulatory frameworks, ultimately promoting a toxic-free environment and minimizing animal use in toxicity testing. At the same time, we acknowledge the current limitations and uncertainties in the application of NAMs for aquatic toxicity.

1.07.P-Mo007 Characterizing Uncertainty in the Disposition of Organic Chemicals in Cellular In Vitro Bioassays

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The potential risk of human and ecological exposures to thousands of chemicals used by society is largely unknown. Traditional in vivo toxicity testing is costly, time-consuming, and there is a need to reduce animal testing, thus driving the development and application of New Approach Methods (NAMs) such as in vitro bioassays. In this context, in vitro-in vivo extrapolation (IVIVE) methods are required to calculate in vivo doses from administered (assumed) in vitro bioassay doses. Calculated in vivo doses can be compared with traditional toxicity data and applied in risk-based contexts by including real-world exposure estimates. Reliable assessments using in vitro data require a comprehensive understanding of chemical distribution within the in vitro test system. One assumption often used in IVIVE dose extrapolation is that the assumed nominal administered in vitro dose directly relates to the response and can be used directly in an in vivo system; however, more than 20 years of experimental and theoretical studies have highlighted that this assumption is invalid for some chemicals and bioassay conditions. Chemical disposition in an in vitro bioassay is a function of the test system parameters (e.g., well-size, cell density, % fetal bovine serum, etc) and the test chemical properties (i.e., octanol-water partition ratio, air-water partition ratio, pKa). In Vitro Mass Balance Models (IV-MBM) help quantify in vitro chemical disposition processes including volatilization, sorption to test materials, and saturation (exceeding solubility limits), and provide estimates of the free and cellular concentrations (CFREE, CCELL). IV-MBM calculations identify when often used assumptions are likely violated and thus aid in vitro data interpretation, application and experimental design to avoid costly errors. This study combines in vitro measurements and IV-MBM calculations to characterize uncertainty in interpreting in vitro bioassay results and to foster confidence in the application of NAMs for chemical assessments. The analysis compares IV-MBM predictions for CFREE and CCELL with available measured data and characterizes the uncertainty in the measured and modelled data as a function of chemical properties and bioassay parameters. General guidance is provided for the expected magnitude of uncertainty when using in vitro

cellular dosing data. Recommendations to better characterize and quantify uncertainty in in vitro dosimetry are outlined.

1.07.P-Mo008 Evaluating Methods for Determining Membrane-Water Partitioning of Surfactants as an Alternative to n-Octanol-Water Partitioning

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The n-octanol/water partition ratio or coefficient (logKow) and n-octanol/water distribution coefficient (log Dow) are key parameters in environmental fate and bioaccumulation assessment of chemicals. Surfactants provide challenges when considering Kow/ Dow since they are amphiphilic structures. Consequently technical difficulties and uncertainties using currently available experimental methods occur due to their specific phase behaviour, complicated by the fact that aqueous solubility is not properly defined for surfactants. In addition Kow/ Dow is poorly representative of the interaction energy of ionic surfactants with organic/ inorganic phases and tissue matrices. The phospholipid membrane-water partition ratio (Kmw) is a promising alternative chemical descriptor for surfactants instead of Kow, as it relates to partitioning into a critical cellular component and accounts for ionic interactions. Kmw can be determined by several experimental methods using artificial phospholipid material. Here we present the results of a study which systematically evaluates three experimental and three computational methods for determining Kmw values for a diverse set of twelve surfactants, covering nonionic, anionic, cationic, and zwitterionic classes. Values derived using experimental approaches using unilamellar liposomes dialysis systems, solid-supported lipid membranes (SSLM) and HPLC columns with Immobilized Artificial Membrane coated silica (IAM-HPLC) are compared with values derived using computational approaches of polyparameter Linear Free Energy Relationships (ppLFERs), Conductor-like Screening Model for Membranes (COSMOmic) and coarse-grained molecular dynamics (MD) simulations. The pros and cons of each method will be discussed in the context of generating reliable and relevant estimates of Kmw for surfactants as a key input into in silico bioaccumulation prediction tools as well as baseline toxicity potential.

1.07.P-Mo009 Effect-Based Screening of Chemical Pollutants Present in Suspended Particulate Matter of German Rivers

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Suspended particulate matter (SPM) plays a vital role in the aquatic ecosystem and can be used to assess the water quality and contamination level. This study employed a panel of effect-based in vitro methods to evaluate the potential toxicity of SPM samples over a 17-year period from two sites at the Rhine River (collected between the years 2005 and 2022), focusing on the presence of chemical contaminants. The samples were extracted by sequential ultrasonication using both a non-polar (hexane:dichloromethane, 1:1) and a polar (methanol) solvent. Both the non-polar and polar extracts were then assessed using effect-based methods, measuring estrogen receptor ? agonistic, androgen receptor antagonistic, aryl hydrocarbon receptor and oxidative stress responses. Notably, polar extracts exhibited high bioactivity 35 out of the 36 tested - in the oxidative stress assay, where the highest bioequivalent concentration was recorded in a sample from Koblenz (2021). The non-polar extracts, on the other hand, showed no oxidative stress response in the concentrations tested. This suggests that polar compounds are driving the oxidative stress response. Initial screening of estrogen receptor ? agonistic and androgen receptor antagonistic activities appears to be affected solely by polar compounds based on the results from a 2015 sample from Koblenz. In contrast, the non-polar extract exhibited a higher activity in the aryl hydrocarbon receptor assay compared to the polar extracts. Ongoing analyses with liquid and gas chromatography coupled with high-resolution mass spectrometry aim to clarify the chemical profiles contributing to these effects. This study highlights the benefits of using effect-based methods as an early warning system to detect chemical hazards in the aquatic environment, as it provides a comprehensive view of water quality assessment.

1.07.P-Mo010 Mechanism-Based Toxicity Screening of Organophosphate Flame Retardants Using Tox21 Assays and Molecular Docking Analysis

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As brominated flame retardants are phased out and regulations on their use become stricter, concerns over organophosphate flame retardants (OPFRs) have increased due to their high production. In response, this study aimed to screen the potential toxicity of emerging OPFRs using in vitro Tox21 assays and in silico molecular docking analysis. For 48 OPFRs collected from the literature, we investigated their bioactivity with human nuclear receptors using Tox21 data, focusing on pathways related to endocrine disruption (ERs, AR), stress response (GR), energy homeostasis (PPARs, FXR), and detoxification (PXR, CAR). For OPFRs not tested in Tox21 assays, molecular docking simulations were performed to predict binding potential. Results showed that CAR/PXR and FXR had relatively high reactivity with diverse OPFRs, indicating potential molecular initiating events (MIEs). Among the 48 OPFRs, 28 interacted with one or more receptors, suggesting they may act as potential stressors of adverse outcome pathways (AOPs) leading to various human diseases. Aryl- and halogenated-OPFRs displayed higher bioactivity compared to alkyl-OPFRs. Additionally, as the logKow value and carbon number of OPFRs increased, their interaction with nuclear receptors also increased. These structure- and physicochemistry-dependent bioactivities provide insights for designing safer OPFRs to avoid regrettable substitutions. Of these prioritized OPFRs, 13 showed low oral points-of-departure (POD) values under 100 mg/kg/day. In contrast, the other 15 OPFRs lacked sufficient data or exhibited less severe toxicity, despite being predicted to be of high concern in our analysis. Since several OPFRs are commonly used in consumer products that can lead to daily human exposure, we suggest that these OPFRs have the potential to reveal undisclosed effects and should therefore undergo further assessment. This work was supported by Korea Environment Industry & Technology Institute (KEITI) through Technology Development Project for Safety Management of Household Chemical Products, funded by Korea Ministry of Environment (MOE)(RS-2023-00215309)

1.07.P-Mo011 Cytotoxicity of Tire-Related Chemicals in Cells from Diverse Fish Species

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Recent studies have shown that 6PPD-quinone, which forms as a result of the transformation of the antioxidant 6PPD in tire rubber, displays marked acute toxicity in some salmonid fish species. Furthermore, similar toxic effects have been observed in cultured cells derived from these fish species. This study aims to evaluate the cytotoxicity of 6PPD and related chemicals, with the goal of identifying safer alternatives, using cultured cells from three salmonid species and two phylogenetically distant fish species, Bluegill (BF-2) and Fathead minnow (FHM). First, to confirm the general sensitivity of each fish cell line to common chemicals, we assessed the toxicity of 3,4-dichloroaniline (3,4-DCA) as a model compound for environmental pollutants. We further evaluated 6PPD and its quinone form, along with other tire-related chemicals. The endpoint was the assessment of cellular metabolic activity measured by the Alamar Blue assay. The results showed no major species-specific differences in the toxicity of 3,4-DCA across the fish cell lines. Additionally, no toxicity was observed for 6PPD in any cell line; however, 6PPD-quinone exhibited toxicity in Rainbow trout (RTG-2) and Coho salmon (CSE-119) cells. This study highlights the species-specific toxicity of 6PPD-quinone at the cellular level by utilizing cell lines derived from phylogenetically diverse fish species. Ongoing evaluation of potential alternative substances will be presented in detail at the poster session.

1.07.P-Mo012 Cytotoxicity Assay on the Fish Cell Line RTgill-W1 to Assess the Acute Toxicity of Nanomaterials

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Nowadays, in vitro methods are seen as a reliable tool to generate appropriate information for the assessment of chemicals and nanomaterials. OECD test guideline (TG) 249 (Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay) has been originally developed for soluble toxicants. The present work aims to assess the applicability of this test to nanomaterials (NMs) by using NMs of different composition, size and shape, and comparing the obtained results with these generated in other fish cell lines and in in

vivo fish tests. For this purpose, the following NMs were investigated: Ag, 10 nm; BaSO₄, 32 nm; Bentonite Nanoclay, 288 nm; CeO₂, 33 nm; Cu, 25 nm; Cu, 50 nm; Cu, 100 nm; CuO, 15-20 nm; CuO, <50 nm; Multi-walled Carbon Nanotubes, length 4.05 µm, thickness 67 nm; TiO₂, 6 nm; TiO₂, 20 nm; ZnO, 34 nm; ZnO, 42 nm. The Nanogenotox protocol was applied to obtain stable dispersions. The stability of the stocks and exposure suspensions was characterized by the measurement of the concentration and size frequency distribution/zeta potential during 24 h exposure period, by ICP-MS or ICP-OES and dynamic light scattering, respectively. The size distribution of the 14 NMs tested was stable during this period. Real concentrations at time 0 were within the 20% range allowed of the nominal concentrations, except for BaSO₄, CeO₂, TiO₂, and ZnO (34 nm). Real concentrations after 24 h dropped significantly for BaSO₄, CuO, and TiO₂ (6 nm) due to deposition. RTgill-W1 cells were seeded in 24-well plates (35×10⁴ cells/well) following the TG249 procedure. L-15ex medium was used for treatment with NMs. A triple cytotoxicity assay (Alamar Blue, CFDA-AM, and neutral red) was performed according to TG249. Potential interferences of NMs with the measures were assessed. The EC50 values obtained for the 14 NMs were similar to those reported in acute toxicity tests in fish and were significantly lower in comparison to other cell lines. Interferences with the NRU assay at the highest concentrations were registered for most of the investigated NMs, and several NMs interfered with AlamarBlue and CFDA-AM.

This study suggests that NMs can be tested following the TG249 protocol, but the stability of the exposure suspension and the possible interference effect needs to be assessed previously. Results also indicated that the assay could predict the acute toxicity in fish, although further data are needed to make a clear conclusion. CSIC Program of Scientific Cooperation with Ukraine Call 2022 and General Protocol of Action for the Assessment of Chemicals and Nanomaterials between MITECO and CSIC.

1.07.P-Mo013 Suitability Of the Rtgill-W1 Cell Line Assay (OECD TG 249) to Predict Acute Fish Toxicity for Surfactants

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The OECD TG 203 fish acute toxicity test is a key requirement in global regulatory frameworks and environmental risk assessment. However, evolving legislation under the Chemical Strategy for Sustainability (CSS) incorporates attempts to reduce vertebrate animal testing. To ensure the continued safety of chemicals, robust, reliable, and regulatory-compliant alternatives must be available. One such option is the RTgill-W1 Fish Cell Line Acute Toxicity assay (OECD TG 249) as a proposed alternative to the fish acute toxicity test. Previously this assay has been shown to have a good correlation with existing in vivo acute fish data for a variety of chemicals. However, the available data are scattered, and despite some surfactants having been tested, a clear applicability domain for this group of chemicals has not been definitively established. Surfactants are used in various home and personal care (HPC), industrial and institutional (HI&I) applications and encompass diverse chemical classes, with variation in alkyl chain length, structure (branched vs. linear), solubility, lipophilicity, functional head-groups, and ionizable potential (cationic, anionic, amphoteric and nonionic), all of which can pose challenges in successfully executing standard (or adapted) test methodologies. Therefore, it is essential to evaluate the suitability of surfactants within in vitro test designs. Here we present initial progress of the work of this Environment and Health Risk Assessment & Management (ERASM) Task Force which aims to address these uncertainties by generating new and/or collating existing in vitro RTgill-W1 data for a selection of non-ionic, anionic, cationic, and amphoteric surfactants. These data will subsequently be compared with existing high quality in vivo and fish embryo toxicity (FET; OECD TG 236) data to provide recommendations and potential limitations around the application of the method for regulatory frameworks and environmental risk assessment of surfactants.

1.07.P-Mo014 Comparing the Sensitivity of Three Rainbow Trout Cell Lines Through Cytotoxicity and Targeted Gene Expression For Aquatic Testing

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The International Organization for Standardization recently approved the RTgill-W1 cell line as an in vitro alternative for acute toxicity testing in fish. While RTgill-W1 reflects acute toxicity in rainbow trout (*Oncorhynchus mykiss*), it represents only one tissue type. Testing with various tissue types can elucidate tissue-specific mechanisms of toxicity that may be overlooked when using a single cell type. In this study,

the rainbow trout cell lines RTL-W1 (liver), RTgut-GC (intestine), and RTgill-W1 (gill) were exposed to a range of 3,4-dichloroaniline (DCA) and cadmium (Cd) concentrations. Cytotoxicity levels and effective concentrations for 50% cell viability (EC50) of Cd and DCA were determined using a multi-endpoint viability assay with alamarBlue, CDFA-AM, and neutral red as markers of cell metabolic activity, cell membrane integrity, and lysosomal integrity, respectively. Results indicated Cd EC50 values of 72.5 μ M, 92.6 μ M, and 234.5 μ M for RTgill-W1, RTgut-GC, and RTL-W1 cells, respectively. For 3,4 DCA, EC50 values were 442.8 μ M, 622.3 μ M, and 653.3 μ M for RTgill-W1, RTgut-GC, and RTL-W1 cells, respectively. Across all cell types, RTgill-W1 was the most sensitive to both 3,4 DCA and Cd, with cadmium exhibiting higher toxicity in all cell lines compared to 3,4 DCA. RTL-W1 cells displayed greater resistance to these chemicals, suggesting that, similar to tissues in vivo, this cell line may possess more robust detoxification mechanisms. To test this hypothesis, cells were exposed to non-toxic and toxic concentrations of cadmium (18.5 μ M and 185 μ M, respectively) and DCA (0.445 μ M and 6.67 μ M, respectively) for 24 hours. Following exposure, total RNA from each cell type was extracted and reverse-transcribed for quantitative PCR (qPCR) analysis. Metallothionein and Cytochrome P450 1A messenger RNA levels will be measured by qPCR as biomarkers of cadmium and DCA exposure and toxicity. With this approach, we aim to assess variability in the sensitivity of different cell types to chemical toxicity and determine whether differences in sensitivity can be explained by molecular detoxification mechanisms across cell types.

1.07.P-Mo015 Applicability of the OECD 249 RTgill-W1 Cell Line Assay for Predicting the Acute Fish Toxicity of Glycol Ethers and Glycol Ether Esters

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Glycol ethers (GE) and GE esters are a group of industrial chemicals typically used as solvents in a wide range of applications. Most are registered under high tonnage bands in EU REACH (> Annex VII), thus require acute fish toxicity data. As part of a voluntary dossier improvement project, the Glycol Ethers REACH Consortium (<https://www.reachcentrum.eu/consortia/glycol-ethers-reach-consortium/>) has been conducting new testing. This involves improving the current acute fish toxicity dataset through strategic testing efforts. Specifically, QSAR predictions and existing OECD 203 data (which demonstrated no or low hazards to fish but often lacked analytical monitoring) were used to prioritize candidate substances for further testing. Subsequently, with the aims of avoiding additional and unnecessary animal testing, OECD 249 was employed to predict acute fish toxicity. Outcomes from the OECD 249 tests were targeted to be used as additional lines of evidence for the acute fish endpoint. For GE substances preliminary OECD 249 results confirmed the previous conclusions that these substances pose no hazards to fish. However, direct comparison of these results to in vivo data demonstrated that the OECD 249 tended to underpredict the toxicity for both GE and GE esters. For example, OECD 249 EC50 values were around 2-fold higher for GE and up to 18-fold higher for GE esters than in vivo LC50 values. Currently, the reasons for this toxicity underprediction are unclear. The OECD 249 has been shown to underpredict the toxicity of chemicals with specific modes of action or substances that are metabolically activated to reactive forms. Moreover, previous studies indicate that there may be differences in enzymatic expression of certain enzymes in rainbow trout gill cells compared to other metabolic organs. Thus, one hypothesis is that diverse toxicokinetics in the in vivo and OECD 249 assays are driving these differences in predicted effect. Overall, this work can be considered as part of the greater available evidence to help define the applicability domain of the OECD 249. Moreover, these findings can be used to inform future testing strategies for other industrial chemistries, and spur further research focusing on deriving fit-for-purpose alternatives to classic vertebrate ecotoxicity tests.

1.07.P-Mo016 Real-Time Chemical Toxicity Monitoring with a Microfluidic Multi-Cell Line Fish-on-Chip Model

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Fish toxicity testing is a crucial component of chemical risk assessment and effluent testing, and there is a growing need for alternative methods to reduce the use of live animals. Fish cell lines can be used as an effective in vitro alternative to predict adverse outcomes. However, most in vitro assays using cell lines are carried out under static exposure conditions and assess cell viability only at a single time point, upon termination of chemical exposure. Thus, no information is gained on how toxicity develops over time. In

this work, we established a microfluidic biosensor for acute toxicity testing under flow conditions in real-time. We employ Electric Cell-substrate Impedance Sensing (ECIS), which measures resistance to an electric current of a cell monolayer on a chip. This label-free and non-invasive technique allows continuous monitoring of cell viability during chemical exposure. We use cell lines from the rainbow trout (*Oncorhynchus mykiss*), a standard species in ecotoxicity testing, of which there are several established cell lines derived from various organs. Using a cell line from the gill, RTgill-W1, we have previously shown that ECIS can be used for time- and concentration-dependent toxicity testing under flow conditions. Moreover, as ECIS is a sub-lethal endpoint, permanent and reversible changes could be distinguished during depuration. This versatile platform has application with chemical toxicity screening as well as the evaluation of water samples. To investigate chemical impacts across multiple organ systems, we have incorporated cell lines from the liver and brain of rainbow trout. We hypothesise that different cell lines may have different sensitivities to certain chemicals. Moreover, by serial connection of channels containing different cell lines, sensitivity of cells in downstream channels may be altered, e.g. by means of biotransformation of chemicals by cells in upstream channels. Thus, by combining multiple cell lines connected in series on the same chip, we make a simple fish-on-chip model for chemical toxicity monitoring.

1.07.P-Mo017 Comprehensive Characterization of Rainbow Trout Hepatic 3D Spheroids: Morphological and Transcriptional Insights

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The demand for alternative ecotoxicology methods is increasing, especially with a focus on New Approach Methodologies (NAMs) to provide reliable in vitro models for chemical hazard assessments. Many existing cell-based assays rely on primary or continuous cell cultures effective for acute toxicity but often lack the necessary complexity for long-term exposure studies. However, few alternative models support comprehensive, long-term toxicity evaluations, essential for understanding chronic impacts. A novel 3D spheroid model derived from rainbow trout (*Oncorhynchus mykiss*) primary hepatocyte (RT-HEP-SP) has emerged as a promising NAM, preserving key morphological, physiological, and biochemical properties for weeks, making it suitable for chronic toxicity studies. However, broader application of the RT-HEP-SP system requires thorough characterization and validation. In this study, high-resolution confocal microscopy was employed to assess various markers, such as spheroid morphology (cytoskeleton, DNA and nuclear integrity, bile canaliculi formation), viability (metabolic activity, hypoxia within the spheroid core), and physiological response (reactive oxygen species [ROS] induction, mitochondrial membrane potential, ethoxresorufin-O-deethylase [EROD] activity, and Phase II metabolism components like uridine 5'-diphospho-glucuronosyltransferase [UGT]) during chemical exposure. RNA sequencing (RNA-seq) and targeted bioassays were used to evaluate RT-HEP-SPs responses to various toxicants. A Biomarker Toolbox was developed with sensitive and reproducible markers for oxidative stress, biotransformation (e.g., aryl hydrocarbon receptor, EROD), and viability (e.g., lactate dehydrogenase). These biomarkers were validated through short-term (24-96 hours) exposures to model chemicals with distinct toxic modes of action (MOAs), including copper, 17 β -ethinyl estradiol (EE2), benzo[a]pyrene, and carbonyl cyanide m-chlorophenyl hydrazone (CCCP), confirming the model's responsiveness and reproducibility. Initial findings indicate that RT-HEP-SP are a reliable model for assessing cytotoxicity, membrane integrity, and oxidative stress, with no core hypoxia detected. RNA-seq analyses reveal their molecular complexity and suitability for the assessment of chemicals with different MOAs. This model shows strong potential as an ecotoxicity screening tool for various chemicals.

1.07.P-Mo018 Development of an Atlantic Cod (*Gadus morhua*) Estrogen Receptor TR-FRET Assay for Assessment of Xenoestrogens in a Non-model Teleost Species

Odd Andre Karlsen, Rhian Gaenor Jakobsen, Fekadu Yadetie and Anders Goksøyr, University of Bergen, Norway

The 3Rs directive (Directive 2010/63/EU), to replace, reduce and refine animal experiments, has been a driving force to move from in vivo studies towards in vitro and in silico approaches. Establishing standardized and validated new approach methodologies (NAMs) is essential to this transition and important for reliable toxicity testing and risk assessment of chemicals. Cell-based luciferase reporter gene assays with ligand-activated transcription factors have been frequently used for chemical toxicity testing and assessment of potential endocrine disrupting properties. Although these assays are sensitive and to some extent adjustable to high-throughput testing, they still suffer from being labour intensive and time consuming. In the recently funded XENOSENSE project, we aim to develop receptor-based molecular tools that are more efficient, less time consuming, and as sensitive as cell-based assays. Time-resolved

fluorescence resonance energy transfer (TR-FRET) has emerged as a promising cell-free technology for assessing the interaction of nuclear receptors with various chemicals. Although some TR-FRET receptor assays are commercially available, these are still limited to model species such as rat and human. In the present work, we have developed TR-FRET assays for the Atlantic cod estrogen receptor alpha (Era). The TR-FRET assay is based on the ligand-dependent recruitment of the steroid receptor coactivator (SRC-1) and uses recombinantly expressed and purified estrogen receptor ligand binding domain. Its performance regarding the sensitivity towards binding of estrogenic compounds is currently evaluated towards a suite of bisphenol A (BPA) analogs, and compared against recently published data obtained with the traditional cell-based luciferase reporter gene assay with the same library of compounds. Our data indicate that Era demonstrate similar ligand-activation profiles in the cell-free TR-FRET assay as in the cell-based luciferase reporter gene assay, but the TR-FRET appears to be more sensitive, producing EC50 values that are between one and two orders of magnitude lower than those obtained for corresponding compounds in the traditional reporter gene assay. Thus, TR-FRET appears as a promising NAM for toxicity assessment that can be established for ligand-activated receptors from non-model species. The authors thank the Research Council of Norway for funding the XENONSENSE project (project no. 342186)

1.07.P-Mo019 Identification and Validation of Biomarkers of Reproductive Toxicity in Fish Cells
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New approach methodologies (NAMs) include in vitro assays that aim to replace animal tests in chemical risk assessment. Because NAMs reduce the reliance on animal data, they are both more practical and ethical compared to traditional methods. Additionally, NAMs may provide more precise mechanistic data and more reproducible results, making them scientifically more desirable. Here we aim to develop NAMs that can replace in vivo fish reproductive toxicity tests in environmental risk assessment with assays that utilise permanent cells of rainbow trout (*Oncorhynchus mykiss*). We undertake this project within the NC3Rs CRACK IT Challenge SAFE. We have previously presented a novel computational pipeline to identify a panel of biomarkers based on mechanistic information of reproductive toxicity in fish. We also identified biomarkers that show robust regulation under reprotoxic chemical exposure as well as poorly studied proteins that demonstrate an association to reproductive signalling pathways in fish. We have now undertaken the first steps towards validating a subset of the identified biomarkers by measuring their expression in the rainbow trout liver cell line (RTL-W1). We exposed the cells to two endocrine chemicals, 17 β -Estradiol and Testosterone, and measured the biomarker expression on the BioMarkHD Fluidigm system in a medium throughput manner. With this approach we identified Insulin-like growth factor 1 (Igf1) signalling as a key mechanistic player. We are currently exposing additional rainbow trout cell lines to a larger panel of endocrine disrupting chemicals to gain more insights into highly regulated pathways in cultured cells, which will form the basis for future endpoints in fish reproductive NAMs. This work is funded by the NC3R CRACK IT Challenge 41 SAFE <https://nc3rs.org.uk/crackit/safe>

1.07.P-Mo020 Using NAMs and Probabilistic Modelling in Component-Based Mixture Risk Assessment: A Case Study on the Risk to Aquatic Environments From Pesticides Causing Mitochondrial Toxicity

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The next-generation environmental risk assessment vision advocates the transition to i) assessing exposure to chemical mixtures, and ii) employing mechanism-based hazard assessment based on new approach methodologies (NAM). Here, we explore the potential of a refined version of component-based risk assessment (CBMRA) methodology, using high-throughput-screening (HTS) bioactivity data combined with a quantitative adverse outcome pathway (qAOP) approach for hazard assessment. The project centres on the quantitative AOP #263, which models growth inhibition through uncoupling of mitochondrial oxidative phosphorylation by chemicals using model aquatic organisms. As a case-study, it uses monitoring data on pesticide concentrations in freshwater from the European Environmental Agency's Pesticide Indicator dataset (Waterbase and WISE statistics). Bioactivity profiles from ToxCast and Tox21 are mapped to an OECD endorsed AOP (#263) to establish equipotent mixture concentrations compared to the model chemical, providing input data to the qAOP model. Uncertainty associated with the different risk components is estimated by statistical modelling, and subsequently integrated and propagated throughout the whole risk characterisation process using Bayesian network modelling. The resulting probabilistic risk characterisation will be compared to risk predictions based on other lines of evidence. This poster presents preliminary results from the application of probabilistic NAM-based CBMRA for this

case-study, addressing conceptual, technical and modelling aspects. It also highlights connections to the European Food Safety Authority's guidance on cumulative risk assessment.

1.07.P-Mo021 In Vitro Comparison of the Sensitivity of Zebrafish to Five Model Marine Fishes to Dioxin-Like Chemicals and Polycyclic Aromatic Hydrocarbons

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Evaluations of the sensitivity of fish when exposed to ubiquitous environmental contaminants such as dioxin-like compounds (DLCs) and polycyclic aromatic hydrocarbons (PAHs) have historically utilized freshwater species. However, studies have shown differences in sensitivity between various freshwater species ranging over 400-fold, highlighting the significant variability within the taxa. Considering the zebrafish (*Danio rerio*) is a popular model freshwater species used as a benchmark for sensitivity assessments, the difference in relative sensitivity compared to marine fish is not currently understood. Thus, the objective of this study was to evaluate the sensitivity of five marine model species, namely the Gulf Killifish (*Fundulus grandis*), Mummichog (*Fundulus heteroclitus*), Inland Silverside (*Menidia beryllina*), Atlantic Silverside (*Menidia menidia*), and Sheepshead Minnow (*Cyprinodon variegatus*). Exposures for each species were performed by conducting a standardized in-vitro aryl hydrocarbon receptor 2 (AHR2) transactivation assay of COS-7 cells transfected with an AHR2 cloned from the species of interest. A diversity of chemical structures were investigated, namely the model DLC, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a chlorinated dibenzofuran (TCDF), a non-ortho and a mono-ortho polychlorinated biphenyl (PCB 77 and 118), PAHs with 5 (BaP), 4 (BAA), and 3 rings (Anthracene), and a methylated PAH (8MBAA). The 50% effective concentrations (EC50) were compared to the corresponding EC50s for zebrafish and the relative sensitivity was calculated. Results suggest that zebrafish AHR2 has different sensitivities to agonists relative to common model marine fishes, with relative sensitivities of the 5 marine species ranging from 1 to 50 times more sensitive. While zebrafish AHR2 might have similar sensitivity to some PAHs, it appears to be substantially less sensitive than the marine fishes to TCDD and TCDF. This further highlights the variability in sensitivity within fishes when exposed to DLCs, as well as the shortcomings of relying on zebrafish toxicity data, particularly for fish families in marine environments. The capacity of in-vitro assays to quickly and accurately generate information for a variety of species, while eliminating the need for largescale lethal sampling, suggest that they are a viable way to fill the current knowledge gaps and ultimately conduct more accurate risk assessments.

1.07.P-Mo022 The Development of a Three-Dimensional Ex Vivo Respiratory Model For Nanomaterial Exposure Using The Air Breathing Organ of *Clarias gariepinus*

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Due to their multifunctional biomedical and industrial applications, nanomaterials, have seen an increase in synthesis, use and waste disposal to the natural environment. The use of copper oxide nanomaterials (nCuO) is emerging throughout various industries including environmental remediation, agriculture and renewable energy, the biodistribution and accumulation of nCuO is however poorly understood in biological organisms. As each characteristic of the nCuO is altered so is the toxicity which leads to a battery of animal testing to ensure safety by design. The African Sharptooth catfish is a niche organism which can respire through an air breathing organ (ABO) which assists it to move between pools when water levels are depleted. A previous study showed nanomaterial uptake specific to the ABO. This study aimed to investigate the interaction, biodistribution and uptake of nCuO in live excised respiratory ABO tissue of *Clarias gariepinus* was compared to a developed three-dimensional model of a similar structure. The ex vivo structures were acutely exposed to nominal concentrations of 350 µg/L and 3.5 mg/L and spiked at 35 mg/L nCuO. Materials were characterized in RO water. Uptake, biodistribution and agglomeration of particles were analysed using CytoViva dark field hyperspectral imaging, light microscopy and scanning electron microscopy and where possible Image J was used to quantify the number of foreign bodies found. Characterization results suggest a dose-dependent hydrodynamic size distribution and agglomeration of nCuO in both live and synthetic tissue. Attachment to the surface of tissue was observed in both groups however the synthetic group statistically resulted in more accumulation and agglomeration. It is hypothesised that might be due to the stability or charge of PLA in water. Toxicology testing of ENM can attempt to include alternate methods, mentioned in this study, as an ethical consideration in reducing animal studies.

1.07.P-Mo023 From Fish Embryo to Fish Early Life Stage – Prediction of effects on growth via effect modelling

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In the context of the environmental risk assessment (ERA) according to the European Union regulations 283/2013 and 284/2013 for plant protection products, numerous animal studies must be performed. Based on the physico-chemical characteristics, for many substances a fish early life stage study (FELS, OECD 210) investigating aquatic chronic effects needs to be conducted to fulfil the current data requirements. This project investigates whether it is possible to predict chronic effects on growth and survival in FELS studies from fish embryo toxicity test data (FET, in acc. to OECD TG 236), with additional length measurements at different time points, by using toxicokinetic-toxicodynamic (TKTD) and Dynamic Energy Budget (DEB) models. DEB models modify growth rate based on an internal energy reserve and translate this growth across maturity stages. A DEB model parameterization for fathead minnow (*Pimephales promelas*) exists in the publicly accessible AddMyPet (AMP) database that we use as a base model onto which we attach a TKTD component to fit the FET growth and survival endpoints. The interaction of TKTD effects in a DEB model enables us to extrapolate from FET results into simulated FELS tests and compare simulated FELS endpoints for growth retardation and survival against existing tests to validate the modeling assumptions and goodness of fit. With this approach a combination of different already existing New Approach Methodologies is used. This is in line with the 3R (Reduction, Replacement, Refinement) principle: Reduction of animal numbers by using embryos for specific ERA questions and Replacement of further animal experiments.

1.07.P-Mo024 Is it safer? Evaluating the Hazards of Bisphenol A Alternatives in Zebrafish Embryos
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Bisphenol A (BPA), a chemical widely used in manufacturing polycarbonate plastics and epoxy resins, is a known environmental and health concern due to its endocrine-disrupting properties and links to reproductive harm and chronic conditions. To address these issues, alternative compounds such as bisphenol AF (BPAF) and bisphenol Z (BPZ) have been developed. However, these substitutes may not be inherently safer, as emerging evidence suggests they can pose comparable or greater risks to human health and ecosystems. To assess whether these alternatives are truly less hazardous than BPA, zebrafish (*Danio rerio*) embryos were exposed to both BPA and its alternatives in two experimental setups. In the first experiment, embryos were exposed to seven concentrations over 120 hours, with mortality, hatching, and malformation rates monitored at 24-hour intervals, alongside a heart rate assay at 48 hours. The second experiment involved exposure to three concentrations below the EC10 for malformations (determined in first experimental setup), following a similar design. At the end of this exposure, behavioural tests and biochemical analyses were conducted, focusing on neurotoxicity, oxidative stress, and energy-related biomarkers. Results indicate that the alternatives tested were more toxic than BPA, with lower lethal concentrations (LC50) values and lower effect concentrations for malformations (both EC10 and EC50). These findings highlight the critical need for continued efforts to identify truly safer BPA alternatives. This work was carried out in the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC) and has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement No 101057014 and CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020) through national funds.

1.07.P-Mo025 Tissue-Specific Responses to a Binary Mixture of Bisphenol A Substitutes in Zebrafish Embryo-Based Bioassays Assessing Estrogenic and Metabolic Activities

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Mixtures of chemicals, notably endocrine-disrupting chemicals (EDCs), represent a major issue as current regulations often underestimate their risks by assessing individual substances only. Most approaches for evaluating EDCs mixtures rely on cellular models, thereby precluding to take into account the complexity of whole-organism systems and capture potential tissue-specific interactions. In this study, we aimed to address this gap by using in vivo transgenic zebrafish embryo models to assess the effects of a binary mixture of bisphenol A substitutes, bisphenol B (BPB) and bisphenol C (BPC), on estrogenic and metabolic pathways in two distinct target organs, the brain and the intestine. Through in vivo fluorescence imaging, we observed that both BPB and BPC induced the expression of the estrogen-regulated aromatase B gene (*cyp19a1b*) in the brain, as well as the intestinal expression of *cyp3a65*, each in a concentration-dependent manner. For each substance and target gene, EC50 values were derived and used to set-up an

experimental ray design for binary mixtures (5 mixture ratios chosen by considering relative potency and 5 exposure levels around the EC50). The observed tissue-specific effects of these mixtures will be compared with predictions from the commonly used concentration addition model for our two differentially regulated target genes. These findings could reveal new insights on mixture interactions in whole-organism systems, across differentially regulated genes expressed in two distinct organs, supporting a better hazard assessment of EDCs mixtures. This work was carried out in the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC) and has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement No 101057014

1.07.P-Mo026 Is There Evidence for Omitting the Water Control in Fish Early-Life Stage Toxicity Tests When Solvents are Used?

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If a solvent is used in the fish early-life stage (FELS) toxicity test (OECD Test Guideline 210), both a water control and a solvent control are currently required. We investigate the feasibility of using only the solvent control and omitting the water control when a solvent is used. The number of fish and resources used in tests for which solvents are required would substantially be reduced by doing so, following the 3Rs principles. A FELS toxicity test database including solvent and water controls and concentration-response data from existing studies was compiled and analysed to determine whether there are systematic differences between the water and solvent controls. The distributions of control data (means, between- and within-replicate variances) for each response variable were investigated for water, solvent and pooled controls. The impact of control choice on the NOEC and EC10 was investigated using FELS concentration-response data. Computer simulations were conducted to determine the potential impact of using only the solvent control on the ECx estimation and NOEC values. The simulations covered the observed ranges of variability and concentration-response shapes for each type of response for fathead minnow, sheephead minnow, and rainbow trout and the solvent dimethylformamide (DMF). In the study, the measurement endpoints including fish length and weight at study end, hatching success, time to hatch, and larval survival were analysed. In addition, ECx values were estimated both by selecting the statistical model and applying model averaging. There is little evidence that an actual solvent effect exists, at least for DMF and fathead minnow. If there is a solvent effect, the scientific literature provides evidence that the solvent effect acts additive to the treatment effect. Simulation studies based on the variability and distributions of responses assessed in the FELS test and the additivity principle show that basing the EC10 or the NOEC on water control data often leads to significant bias, whereas basing these on the solvent control is relatively free of bias. Altogether, there is preliminary statistical evidence to support omitting the water control in FELS studies using a solvent. The views, conclusions, and recommendations expressed in this presentation are those of the authors and do not necessarily represent the policies or positions of their affiliated organisations.

1.07.P-Mo027 Comparative Effect Assessment of Bisphenol Analogs by High Content Analysis in Zebrafish Embryos

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Bisphenol A (BPA), a common bisphenol plastic additive, has been banned from certain products because of its endocrine-disrupting properties. As a result, substitutes such as BPE, BPG, BPAP, and BPZ were developed and are in use. Many of these substitutes are not thoroughly characterized and may have similar biological activities as BPA. Using a high content screening assay a comparative and comprehensive assessment of BP substitutes was conducted for the zebrafish embryo model with the focus on non-endocrine (neuro)developmental effects. In contrast to previous analyses in the zebrafish embryo, the comparative effect assessment was based on an automated image and video analysis approach reducing the observer bias and increasing the reproducibility of results. The effects were quantified using behavior endpoints, heart rate assessment and quantification of deviation of morphological endpoints from controls. For all endpoints concentration-response modelling was applied to derive effect concentrations. The lack of swim bladder was the most sensitive endpoint for all of the tested bisphenols, particularly for BPE. The specificity of responses was estimated by applying the baseline toxicity concept and calculating effect ratios. Moreover, the effect profile of bisphenols was compared with 2 baseline toxicants and a screening approach to prioritise further bisphenol A analogues for detailed assessment was developed. Comparison to baseline toxicity indicated that the developmental effects were primarily driven by unspecific baseline toxicity with BPE representing the most specific compound. Cluster analysis on effect ratios supported

that BPE was more specific than the other BPA substitutes, also in terms of the number of affected endpoints. The study was supported by the EU projects PARC and PrecisionTox. This work received funding by the European Union's Horizon 2020 research and innovation program project Toward Precision Toxicology: New Approach Methodologies for Chemical Safety (PrecisionTox), grant agreement no. 965406 and the project "European Partnership for the Assessment of Risks from Chemicals (PARC)", grant agreement no 101057014.

1.07.P-Mo028 Assessing Contaminant Sensitivity in Early Life Stage Fishes using Transcriptomic Points of Departure

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Chemical regulatory assessors require hazard data from multiple species spanning a range of sensitivities to develop environmental quality guidelines. Due to lengthy exposures, high costs, and ethical concerns, chronic chemical hazard data for diverse species are currently lacking. New Approach Methods (NAMs) have been touted as potential alternatives that could provide toxicity data quickly and more ethically. Our group is developing a NAM that uses early life stage fish exposures to obtain transcriptomic points-of-departure (tPODs) for environmental chemicals. Previous studies support the possibility of substituting chronic apical PODs (aPOD) with tPODs generated from short-term exposures given their high degree of correlation. The overall objective of our project is to validate tPODs as suitable toxicity data for the derivation of environmental quality guidelines. As a first step, we assessed the reliability of tPODs to predict sensitivity to 6PPD-Quinone (6PPD-Q), and Bisphenol A (BPA) in several listed and model fishes in an early life stage test. Eleutheroembryos (0 to 24 hours post-hatch) were exposed to test chemicals for 24 hours at 12 different concentrations. This concentration range spanned 9 orders of magnitude in an attempt to capture both transcriptomic and organismal level responses. RNA was isolated from pooled eleutheroembryos, followed by RNA sequencing and data processing using ExpressAnalyst. Our current results show that species differences in sensitivity at an organismal level are reflected in variability in tPOD values. Future work will focus on comparing tPODs across fish species relative to chronic apical endpoints, paving the way for the potential use of tPODs in guideline development for chemicals of concern.

1.07.P-Mo029 EMERGE, a New Transgenic Zebrafish Embryo-Based Assay to Assess Metabolic Disrupting Chemicals (MDCs) in the Intestine

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Metabolic disrupting chemicals (MDCs) are increasingly associated with metabolic disorders such as obesity, type 2 diabetes and hepatic steatosis. To identify MDCs, new approach methodologies based on mechanisms of action are needed. Despite the intestine's key roles in metabolism, gut disruption by MDCs remains poorly studied compared to the alteration of liver and adipose functions. To address this gap, we developed EMERGE (Effect of Metabolic Endocrine disruptors in Gut of zebrafish Embryos), a new bioassay based on a zebrafish transgenic line (tg(cyp3a65 :GFP)). This transgenic line expresses GFP under the cyp3a65 promoter, an ortholog of human CYP3A4, a cytochrome involved in both endogenous and exogenous metabolisms of the liver and the intestine. CYP3A4 expression is regulated by several transcription factors, including the pregnane X receptor (PXR) and aryl hydrocarbon receptor (AhR), which are key mediators of xenobiotic responses and detoxification process. The tg(cyp3a65:GFP) zebrafish line was created using Tol2 transgenesis to visualize cyp3a65 responses to MDCs exposure. Embryos were exposed to sublethal concentrations of chemicals, followed by fluorescence imaging of the intestine, GFP quantification and data analysis. The bioassay was validated using zebrafish PXR and AhR agonists, confirming concentration-dependent induction of GFP expression correlated with cyp3a65 mRNA levels.

Subsequently, screening of 22 suspected MDCs from diverse chemical families (e.g bisphenols, biocides, pharmaceuticals) revealed varied effects : 14 induced, 4 inhibited, and 4 had no impact on GFP expression. The assay also highlighted the potential role of additional regulatory pathways (e.g glucocorticoid receptor) and demonstrated a good reproducibility across independent trials. EMERGE offers a sensitive and robust tool to screen the presence and/or the effects of MDCs complementing existing models and providing new informations on chemicals property to target the intestine. To confirm the relevance of the assay for human health assessment and further regulatory validation and

implementation, comparison of our results with MDCs effects on cyp3a4 through mammalian systems should be interesting. This study was funded by the H2020 OBERON project (grant agreement no. 825712).

1.07.P-Mo030 Applicability of the Fish Plasma Model to the Wide Pharmaceutical Landscape: Influence of Physico-chemical and Pharmacokinetic Parameters in Two Fish Species

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The fish plasma model (FPM) is a theoretical model to predict the concentrations of pharmaceuticals in the blood plasma of exposed fish, based on the lipophilicity of the pharmaceutical. By comparing to effective pharmaceutical plasma concentrations in humans, the risk for unintended effects in exposed fish can be assessed without conducting animal testing. Within the research project PREMIER (Priorisation and Risk Evaluation of Medicines in the Environment), the FPM was challenged by application to substances that to date are underrepresented in FPM testing. Based on their physico-chemical and pharmacokinetic parameters and the margin between available chronic fish toxicity and the predicted API concentration in fish plasma, the following substances were selected: quetiapine hemifumerate (an anti-psychotic, cationic at pH 7), olaparib (an anti-neoplastic, not ionizable) and mycophenolic acid (an immunosuppressant, anionic at pH 7). All three compounds were investigated in adult male and female fathead minnow (*Pimephales promelas*) and sub-adult female rainbow trout (*Oncorhynchus mykiss*). The exposures were performed for 14 days in flow-through systems with the respective APIs dosed via water. Each exposure involved three test concentrations per substance and a control. The test concentrations were selected to avoid any possibly toxicological effects whilst seeking to maximize internal API concentrations for enabling analytical chemistry. Fish were sampled at days 10 and 14. For all three investigated compounds, analytical methods were developed, validated and applied to the various sampled matrices (water, blood plasma and body tissue for each fish species). All exposure experiments have been successfully completed. Lack of any substance-related apical effects in the exposed fish and the ability to quantify the target substances in most samples confirm the appropriateness of test concentrations. The evaluation of data is currently ongoing and will be presented at the SETAC meeting. Preliminary analyses indicate differences in plasma concentration between male and female fathead minnow as well as differences between the two fish species. Pharmacokinetic parameters underlying differences in API concentrations in blood plasma between test compounds and test species, as well as departures from API concentrations predicted by the FPM are being determined. Ongoing quantification of API metabolite concentrations in blood plasma is helping in this regard.

1.07.P-Mo031 Expanding the Applicability Domain Of Fish Cell Lines for Measuring the Bioaccumulation Potential of Zwitterionic Pollutants

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Scientific and ethical constraints associated with in vivo bioaccumulation assessments with fish have triggered the development of animal alternatives, such as cell-based systems. Among these systems, fish cell lines have been previously highlighted as suitable models to estimate bioaccumulation parameters, such as bioconcentration factors (BCFs), by assessing chemical partitioning between e.g., culture medium and the intracellular compartment. While most of the existing data using this approach have been generated for neutral organic pollutants, previous studies have successfully employed fish cell lines to assess the bioaccumulation of a few anionic and cationic compounds. However, molecules with different structural properties (e.g., hydrophobic tail lengths) and that display both positive and negative charges (i.e., zwitterions) may undergo complex uptake and distribution patterns that influence their bioaccumulation dynamics. Therefore, to further explore the applicability domain of fish cell lines in conducting bioaccumulation studies for different chemical classes, we here explore the bioaccumulation potential of four zwitterionic surfactants: (Lauryldimethylammonio)acetate and the analogs N,N-Dimethyl(C10-C14)amine N-oxides, using rainbow trout (*Oncorhynchus mykiss*) cell lines. Cytotoxicity assays using the gill cell line RTgill-W1 indicated that these zwitterions do not compromise cell viability at concentrations lower than ~1.5 µM, allowing for bioaccumulation tests to be conducted at environmentally relevant levels (e.g., less than 0.5 µM). As bioaccumulation data for these chemicals is limited, the on-going work employing gill (RTgill-W1) and liver (RTL-W1) cell lines aims to provide important insight about their bioaccumulation potential, thus contributing important evidence to the environmental risk assessment of zwitterionic substances.

1.07.P-Mo032 In Vitro Hepatic Biotransformation Assays for Pesticides in Livestock: Opportunities and Limitations

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To date, human safety testing for novel pesticide active substances involves a series of different in vivo studies in vertebrates, including livestock studies to assess residue levels in edible commodities.

According to OECD Test Guidelines 503 and 505, at least one lactating ruminant and one laying poultry species must be examined. Mechanistic data on the metabolism and kinetics of pesticides in these species is, however, limited, but would be crucial for reliable early-phase predictions aiding the development process and species extrapolations, for example to other bird and mammal wildlife species in the context of in silico ecotoxicological risk assessment. In line with the ongoing efforts to replace, reduce and refine animal testing (3R principles), new approach methodologies (NAMs) could address these data gaps regarding livestock metabolism and kinetics. Metabolically relevant in vitro systems, like rainbow trout sub-cellular liver fractions or rat primary hepatocytes, have already gained regulatory acceptance for specific applications, including intrinsic clearance and comparative metabolism studies. This study aimed to validate an in vitro model for livestock metabolism by evaluating the capacities and limitations of sub-cellular liver fractions from the model species, goat (*capra hircus*) and hen (*gallus gallus*). For this purpose, two well-characterized pesticide active substances, labelled with radioactive ¹⁴C, were applied to the test systems following initial metabolic characterization. After incubation in a shaking water bath, supernatants were analyzed using high-performance liquid chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS). Recoveries and mass balances were determined by liquid scintillation counting and selected samples subjected to radio-HPLC. The evaluation of the metabolic profiles, including metabolite identification, revealed that the majority of biotransformation products detected in vivo were also formed in vitro. The qualitative overlap was, however, substance-dependent, and the quantitative ratios of the metabolites did not match the in vivo situation in most of the cases.

Consequently, while sub-cellular fractions are suitable for qualitative metabolite screening, they provide limited quantitative information on livestock metabolism. Future studies may address some of these limitations by using more physiologically relevant in vitro systems, such as primary hepatocytes.

1.07.P-Mo033 A Novel Multiplex Bioassay Technology for Detection of Emerging Contaminants in Water

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Environmental pollutants, such as pharmaceuticals, personal care products, and pesticides, are a growing concern globally due to their potential adverse effects on aquatic ecosystems and human health. Water quality analyses to assess their risks are generally based on a combination of chemical and biological analyses. Yet, these methods are often resource-intensive and time-consuming in practical application. To implement effective measures for improving water quality and assess risks to humans and the environment, quick, comprehensive, and cost-effective methods are required. In this project we aim to develop and validate a novel multiplex biosensor technology. The technology, called receptomics, is based on a flow cell with a printed array of hundreds of receptors in a square centimeter, which are expressed on a surface of living human cells (HEK293). The results are obtained based on light signals (fluorescence or luminescence). Upon binding of the pollutant (ligand) to the receptor, the receptor is activated, resulting in Ca²⁺-triggered luminescence or fluorescence. The emitted light signal is then captured by a microscope (www.receptomics.com). Since the assay is flowcell based it allows for a measurement every 5 minutes. Previous studies have demonstrated that human hormone receptors, such as human estrogen, androgen, nuclear pregnane X receptors, can bind water pollutants like pesticides, PFAS and hormones. These receptors will first be tested in the receptomics setup using pure ligands and at a later stage this approach can also be used to study complex water samples. The receptomics technology can support water managers and drinking water companies to continuously monitor water quality, ensure it remains at a high level by providing target compounds to mitigate. Moreover, the developed system can also be used as an early warning system and as a screening method in the development of risk-driven measurement programs in the (drinking) water sector. We will present our first results using this bioassay for water quality assessment.

1.07.P-Mo034 Endocrine Disruptive Potential of Chemical Mixtures From Settled Dust Samples Collected in Seven European Countries

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Humans are continuously exposed to complex chemical mixtures, with indoor environments being a significant source due to prolonged occupancy, limited ventilation, and the presence of chemical sources. Many chemicals found indoors exhibit endocrine-disrupting properties, which may pose health risks. This study employed a battery of NAMs in vitro bioassays to assess the endocrine-disrupting potentials of complex chemical mixtures extracted from indoor settled dust samples sampled across households with small children in seven European countries (Czech Republic, Estonia, Italy, Netherlands, Portugal, Slovenia, and UK). The battery consisted of human cell-based reporter gene assays, namely AZ-AhR model for Aryl hydrocarbon receptor (AhR)-mediated effect, HeLa9903 for estrogenicity and anti-estrogenicity, MDA-kb2 for androgenicity and anti-androgenicity and PZ-TR for thyroid hormone receptor-mediated effects. The battery detected some endocrine-disrupting activities in most of the samples. However, the study revealed diverse effect patterns, the intensity of the effects, and the main modes of action of the chemical mixtures associated with settled dust among households from different parts of Europe. Namely antiestrogenic and AhR-mediated effects were frequently detected across the studied countries. To contextualize these findings, observed bioactivities will be compared with data from iceberg modeling obtained using available toxicity potencies based on available data from the target and non-target chemical analyses. The pervasive endocrine-disrupting activity in indoor environments and the challenges in identifying responsible pollutants highlight potential health risks associated with indoor exposure. This project received funding from the European Commission Horizon Europe, HADEA, research and innovation programme under grant agreement Project No 101057499 - INQUIRE.

1.07.P-Mo035 Systematic Evaluation of High-Throughput Toxicokinetic (HTTK) Model Predictions For The Terminal Elimination Half-Life (HLT) in Humans

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Quantifying toxicokinetic (TK) processes is essential for chemical assessments. New Approach Methods (NAMs), such as in vitro-in vivo extrapolation (IVIVE) and high-throughput toxicokinetic (HTTK) modelling, are instrumental in generating TK data required for chemical priority setting, screening, and risk assessment. However, there is a critical need to systematically evaluate existing data and NAMs to build confidence in their application for scientific evaluations and decision-making. This includes defining the applicability domain (AD) and characterizing the uncertainty of IVIVE and HTTK models. One TK parameter required for IVIVE and other chemical assessment objectives is the whole-body total (terminal) elimination half-life (HLT), which relates directly to the terminal elimination rate constant (k_T) and total clearance (CLT). Various HTTK models and their necessary input data are incorporated in the free online Exposure And Safety Estimation (EAS-E) Suite platform (www.eas-e-suite.com). These models can be used to calculate HLT and other TK parameters and include (i) multiple Quantitative Structure-Activity Relationship (HLT-QSAR) models that have been validated following OECD QSAR developmental guidance, ii) the EAS-E Suite one-compartment physiologically based biokinetic (1Co-PBK) model, which can be parameterized to simulate TK for different mammals, and iii) the 3 Compartment steady-state model developed by the US EPA and implemented in the "httk" R package. These different models were applied to more than 15,000 discrete organic chemicals to assess the AD of each model and to compare their predictions against (i) available measured in vivo HLT data from humans ($n \sim 1,000$) and (ii) each other. There are strong positive correlations (e.g., $r^2 = 0.5-0.9$) for the HLT predictions between the 1Co-PBK, HLT-QSAR and in vivo HLT data. There are weak positive correlations (e.g., $r^2 < 0.2$) for the HLT predictions between EPA httk model and the other types of models and in vivo HLT data. However, most HLT predictions for the same chemical are within about an order of magnitude providing opportunities for consensus based modeling which considers uncertainty and assumptions in the various modelling approaches. The results provide some guidance for the application of IVIVE and HTTK models in chemical evaluations. Future research should seek to better understand why the models give highly divergent predictions for certain chemicals.

1.07.P-Mo036 The Parhyale hawaiiensis Testing Platform as a Potential Eco-NAM

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Eco New Approach Methodologies (ECO-NAMs) are of growing interest in the ecotoxicology community. Invertebrates are being accepted under REACH requirement as alternative methods, e.g., bioconcentration in the *Hyalella azteca*. As a step forward in reducing testing with vertebrate model, this work aims to introduce a testing platform using the *Parhyale hawaiiensis* model organism. This amphipod

has a circumtropical distribution and inhabits coastal areas. As it is epibenthic, it is possible to evaluate water and sediment, including via feeding. Its genome (3.6 giga bases) has been already sequenced and the animal is explored as a model in EVO-DEVO studies. Organisms collected from the field in 2010 have been maintained in the laboratory. They are fed with fish food 4 times a week, maintained at 26°C, 12h photoperiod, salinity of 30 g/L. Several substances were used as proof of concept and hazard characterization including benzo[a]pyrene, dyes from natural origin (emodin, alizarin, dermocybin, dermorubin) or synthetic (disperse and direct azo class), metals (Cd, Cu, Zn, Ag), 3,4-Dichloroaniline, pesticides (diflubenzuron), 6PPD-quinone and Ag based nanomaterials. In the laboratory, miniaturised assays were conducted to verify acute and chronic toxicity. Toxicokinetics studies performed in the lab using hemolymph internal doses were conducted for metals (Ag), dyes and PFAS. Differential gene expression was explored after exposure to metallic nanomaterials (Ag). PFAS bioconcentration factors, were determined in the hemolymph. Comet and micronuclei assays were established in the hemolymph. Sperm cell viability and comet assay were developed. For field studies a cage was constructed, and adults survived at least for 96h when placed in two sites with low levels of contamination. The concentrations of PFOS, Br- PFOS and PFOA, in the hemolymph of those organisms, were <LD. Studies in higher contaminated sites are being conducted. We believe that the Parhyale testing platform is a powerful tool for both lab and field testing. Protocols for immunotoxicity, behavior, and embryo abnormalities are being developed. Metabolic capacities studies are being conducted to improve toxicokinetic information. More substances should be tested in this platform to confirm its utility, but the results obtained so far are promising making *P. hawaiiensis* an attractive potential NAM for future applications. The authors thank FAPESP (2023/10693-5), CNPq and CAPES code 001.

1.07.P-Mo037 Ecdysone Receptor Agonism in S2 cells for Insect-Specific In Vitro Assay Development

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The traditional approaches to Environmental Risk Assessment (ERA) of chemicals are generally limited to in vivo exposure studies assessing only apical endpoints of a small number of representative model species serving as proxies for entire ecosystems. To better address the heightened challenges posed by the increasing variety of chemicals entering the environment - and to align with the evolving regulatory and ethical landscape towards reducing or eliminating animal testing - New Approach Methodologies (NAMs) introduce a more mechanistic framework for ERA. The operationalization of NAMs relies on the development and validation of in vitro and in silico approaches, including the use of Adverse Outcome Pathway (AOP) frameworks, which represent the foundational elements of mechanistic-based next-generation ERA. This project aims to develop an in vitro assay based on the Ecdysone Receptor (EcR) Agonism AOP using *Drosophila melanogaster* and its embryonic (S2) cell line. Ecdysone is a hormone responsible for development and metamorphosis of insects and is often targeted in pesticide development as a highly specific insecticide. However, EcRs are also found in a range of non-target species, such as pollinators, highlighting the need for a more protective ERA, and a deeper mechanistic understanding of the pathways involved. The first step towards this goal is characterising the molecular response to Ecdysone in the S2 cell line. In vivo, an Ecdysone pulse triggers a genetic cascade that initiates moulting, a process that requires both the presence and the subsequent removal of the hormone for full completion. Therefore, continuous exposure to an EcR agonist was incorporated into the AOP. This work investigates two exposure scenarios in vitro: the relevant (short-term) exposure scenario, imitating a pulse of ecdysone, and continuous exposure scenario. Results demonstrate the in vitro model can illustrate the initial key events of the EcR Agonism AOP as well as mimic the natural events in vivo. This project will be taken a step further delving into the adverse effects in vivo, including morphology of EcR agonism as well as the molecular events underlying the processes.

1.07.P-Mo038 High-Throughput Assessment of Pesticide Toxicity Using *C. elegans*: Impacts on Growth and Life Stage Development

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C. elegans is a versatile and efficient model for toxicity testing across diverse endpoints, leveraging its transparency, rapid growth, and compatibility with high-throughput formats. Its well-characterized genetic and signaling pathways, extensive mutant libraries, and CRISPR adaptability enable mechanistic studies with relevance to both human health and ecological systems. To assess the value of a high-throughput assay (HTA) system, we tested 60 pesticides, evaluating its potential to complement the current battery of human health and ecological models. The results highlight opportunities for enhancing predictive

capabilities and broadening the applicability of toxicity screening frameworks. Partial funding for this project came from Bayer AG.

1.07.P-Mo039 New Approach Methodologies (NAMs) for Identifying Endocrine-Disruption Using the Model Organism *Caenorhabditis elegans*: The Case of Bisphenol A Alternatives

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Many alternative compounds to bisphenol A (BPA) have already shown to be regrettable substitutions because they also demonstrate endocrine disrupting properties. The European Partnership for the Assessment of Risks from Chemicals (PARC) therefore uses the BPA alternatives as a case study to address shortcomings in regulatory approval processes, e.g. by developing New Approach Methodologies (NAM). To bridge the gap from in-vitro to suitable, whole organism testing approaches with a focus on endocrine disruption, *Caenorhabditis elegans* emerges as a promising model. Its transparency, short lifespan (3 days @ 25°C), well-known genome, the comparably high degree of vertebrate gene homology makes it a suitable organism for NAM development. We use *C. elegans* to combine the apical test endpoints growth, reproduction and behavior, with molecular effects at gene transcription level. The focus is on endocrine effects and the elucidation of underlying mechanistic key events. BPA alternatives, such as Bisphenol E and AP, for which limited data exist, were tested in liquid medium either in 96 well plates with one worm per well and up to 10 worms per concentration or in cell culture flasks. As reference substance and to compare with literature results, tests with BPA were also conducted. Around 1000 worms were pooled per sample to gather enough material for RNA extraction and subsequent long-read RNA Sequencing and qRT-PCR. First results on growth, reproduction and behavior (head thrashes) showed effects only at concentrations above environmentally relevant levels. In contrast, molecular endpoints are more sensitive and instrumental for the identification of key biological pathways involved in endocrine disruption and other modes of action caused by BPA alternatives. Possible next steps in the development of *C. elegans* based NAMs may be to study metabolism changes or explore the use of fluorescent reporter lines for the key pathways identified. Overall, the potential of *C. elegans* as an alternative invertebrate animal model for testing endocrine disruptors is being evaluated.

1.07.P-Mo040 Automatization of a Sea Urchin Teratogenicity Test (STT) to Improve Marine Assessment of Chemicals and Mixtures

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The marine environment faces increasing pressure from chemical pollutants originating from various sources, including industrial discharge, agricultural runoff, and pharmaceutical waste. Understanding the potential impact of these chemicals on marine organisms is crucial for ecosystem health. Traditional toxicity tests often focus on lethality, but sublethal effects on development can have significant long-term consequences on populations. Sea urchins offer a powerful model for evaluating the developmental toxicity of marine pollutants. Their external fertilization and transparent embryos allow easy observation of morphological abnormalities. Sea urchin embryo developmental tests, including larval development assays, and assessment of skeletal formation, can provide valuable insights into the mechanisms of toxicity [1]. Nonetheless the observation and measurement of these parameters under a microscope is time consuming, operator dependent and poorly compatible with industry needs for chemicals screening. Here we present an automated version of STT through automated image analysis. In a first step we evaluate the dynamic of the response obtained after the exposure to reference compound. Then we address the interest of sea urchin teratogenicity test in a more global testing strategy by comparing the impact of different mixtures four marine organisms: bacteria (Microtox assay), Diatoms (ISO 10253:2024), corals [2] and our automated sea urchin larval developmental test. Here we present an automated version STT. This method allows to test up to 40 chemicals in parallel, to limit the operator subjectivity, and is compatible with high throughput screening strategy for both raw materials and formulas. We propose to include this test in a global strategy to assess the potential impact of formulas on the marine environment and support the relevance of environmental claims.

1.07.P-Mo041 Developing High Throughput Metametabolomics in Freshwater Periphyton to Enhance Chemical Risk Assessment

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The increasing chemical pollution of aquatic ecosystems presents a challenge in linking chemical exposure to impacts at higher biological levels. While various descriptors exist for individual and

population-level effects, assessing impacts at the microbiome community level remains underdeveloped. Aquatic periphyton, a diverse microbial assemblage critical to ecosystem functions, is emerging as a model for studying ecological impacts on microbiomes. Among available techniques, metabolomics enables simultaneous characterization of exposure, associated effects and toxicity pathways, offering a promising approach to detect early, sensitive responses of microbiomes to chemical stress. However, applying metabolomics to aquatic microbial communities (i.e., metametabolomics) is still limited by its relatively low throughput for screening. Therefore, this work aims to develop a high throughput workflow with miniaturized exposure setups, automated sample preparation, data acquisition, and data analysis to enhance chemical risk assessment in aquatic ecosystems. Periphyton samples (0.5–10 mg dry weight) were analysed to determine the minimum quantity for accurate metabolomics profiling. Our results showed no marked differences between 1 to 10 mg, while 0.5 mg displayed distinct differences from other quantities. Metabolite intensities were higher for 0.5 mg and 1 mg compared to 5 mg and 10 mg, likely due to reduced matrix effects. Therefore, 1 mg was considered as an optimal balance, providing sufficient intensity and comparable results across different matrix quantities. By testing different microplates and glass discs (48, 24 and 12-well) for periphyton colonization, we found that different size of discs led to similar periphyton quantity after 14 days, averaging around 1 mg. Thus, 48-well size discs and microplates will be further used for the exposure setup. Automated extraction is under development. A standardized data analysis pipeline will be developed to determine community metabolism sensitivity threshold based on aggregated metametabolome dose response (DRomics) and metametabolome annotation. This workflow will soon be applied to screen pesticides and pharmaceuticals, addressing regulatory gaps in evaluating chemical toxicity on microbial communities and their impact on aquatic ecosystem functions.

1.07.P-Mo042 Application of Electric Cell-Substrate Impedance Sensing (ECIS) Technology in the Ecotoxicological Evaluation of Industrial and Wastewater Effluents

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This study utilizes Electric Cell-Substrate Impedance Sensing (ECIS) to evaluate the ecotoxicological impacts of wastewater effluents. Samples were collected from industrial raw influents, treated effluents, influents and effluents from wastewater treatment plants (WWTPs). Cytotoxicity assays were conducted using human HepG2 and rainbow trout RTgill-W1 cell lines, alongside acute aquatic toxicity tests using fish and daphnia. The biological response data were further integrated with water pollutant analyses to evaluate the correlation between toxic substances and biological responses. Correlation analysis was applied to determine the toxic units (TU_a) derived from cell-based assays and acute aquatic toxicity tests. Results demonstrated a significant correlation between RTgill-W1 cell responses and acute fish toxicity outcomes, highlighting the potential of the RTgill-W1 cell line as a promising in vitro alternative to acute fish toxicity test. Furthermore, RTgill-W1 cell line in ECIS system exhibited higher sensitivity to pollutants compared to HepG2 cell line, indicating its superior applicability for detecting waterborne toxicants. We further compared bioassay results with pollutant analyses, revealing correlations between toxicity responses and certain water pollutants (e.g. ammonia). Moreover, daphnia acute toxicity demonstrated a correlation with other water pollutants, including heavy metals, indicating species-specific sensitivity to different pollutants. The variability observed between bioassays highlights the importance of a multi-species approach for comprehensive ecotoxicity evaluation, as each organism may respond differently to specific contaminants based on their unique physiological and metabolic pathways. These findings further underscore the feasibility of real-time biological monitoring systems for detecting water pollution using live-cell systems. Traditional water quality monitoring primarily relies on physico-chemical parameters, which may fail to capture the dynamic interactions between pollutants and living organisms, such as synergistic, additive, or antagonistic toxic effects. Incorporating biological endpoints, such as cell-based toxicity data, into water quality surveillance systems can fill this gap, offering a more holistic, real-time assessment of water quality and environmental health.

1.07.P-Mo043 New Approach Methodologies To Evaluate The Toxicity Of Anticoagulant Rodenticides In Target And Non-Target Organisms

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Anticoagulant rodenticides (ARs) are widely used to control rodent populations. Nevertheless, there is a potential risk for the environment and non-target organism derived from their use. The aim of the present study, as part of the PECAURA project, was to establish a protocol to be further applied to alternative substances. Comparisons among data from new approach methodologies (NAMs) and in vivo studies in model species were performed. This allowed to evaluate the usefulness of NAMs to check the toxicity of six ARs (brodifacoum, bromadiolone, chlorophacinone, coumatetralyl, difenacoum and warfarin). In vivo

data (LC/D50) was collected from literature for a target, *Mus musculus*, and non-target species, *Oncorhynchus mykiss* and *Daphnia magna*. Cytotoxicity (EC50) was evaluated checking for mitochondrial, plasma membrane or lysosomal activity effects in murine (Hepal-6) and rainbow trout (RTH149) hepatoma cell lines after exposure to a range of concentrations (0.78-100 mg/L) for 24, 48 and 72 h. In vitro and in vivo data were categorized according to their toxicity, following the Regulation (CE) n° 1272/2008. Computational biology methodology was applied as a predictive tool of binding affinities of the three species vitamin K epoxide reductase (VKORC1) and ARs (AutoDockVina, PyRx 0.9.8 package). For *M. musculus*, in vitro and in vivo toxicity data, together with the docking analysis, suggested that the ARs leading to toxicity in cells and animals had a higher binding affinity (BA) to the VKORC1. This was observed after any time of exposure in Hepal-6. For fish, in vivo and in vitro data did not follow the same pattern. Indeed, bromadiolone, coumatetralyl and warfarin did not show cytotoxicity (24h), but they were toxic to animals. However, bromadiolone turned into cytotoxic after 48 h. In this case, coumatetralyl and warfarin showed the lowest BA, being also the less toxic ARs in vivo and in vitro. Even when the BA pattern is similar for both species, values are lower for *O. mykiss*. In this sense, the higher toxicity showed by both molecules in fish denotes their higher sensitivity. For *D. magna*, it was also possible to establish an association between in vivo and in silico data for all ARs. The differences between the species should be considered for ecological risk assessment, and in silico data should be interpreted case-by-case. Funding: Project TED2021-131186B-I00 supported by MCIN/AEI/10.13039/501100011033 and the EU NextGenerationEU /PRTR.

1.07.P-Mo044 An Integrated Testing Strategy (ITS) for Acute Fish Toxicity: Fish Embryo and RTgill-W1 Cell Line with Threshold Approach

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Acute fish toxicity (AFT) tests typically use mortality as the endpoint to assess the toxicity of environmental pollutants. However, current regulatory policies require a large number of fish for testing. In recent years, in vitro alternative methods, such as fish embryo toxicity test (FET) and fish cell line toxicity (FCT) tests, have been developed. However, no single alternative test can fully replace traditional animal testing. To address this, an ITS has been proposed to combine various alternative methods effectively, supporting regulatory decision-making. These alternative methods have demonstrated a certain level of predictive ability. Therefore, we aim to establish an ITS for predicting AFT, coupled with a threshold concentration (TC) method, to reduce the need for fish in testing. We collected data from the literature to determine the correlation between alternative tests and fish tests, as well as the feasibility of using ITS. Additionally, we selected common heavy metals and organic pollutants found in wastewater to perform alternative tests for external validation of ITS. The results showed that NAMs have significant correlation with AFT test. Combining QSAR Toolbox and TC methods aids in preliminary toxicity screening and reduces the likelihood of false negatives in predictions. The predictive accuracy of ITS for AFT reaches 74-90%. Additional wastewater-related substances were selected for external validation of ITS, achieving a predictive accuracy of 66.7-88.9%. We developed four ITS using NAMs to predict fish toxicity. All the strategies demonstrated a high predictive power. Users or institutions can select from four ITS, each optimized for flexibility, animal welfare, or sensitivity, to predict fish toxicity.

1.07.P-Mo045 In Silico Prioritization of Water Micropollutants for Quantification of Their Contribution to the Aryl Hydrocarbon Receptor (AhR) Mediated Toxicity

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The complexity of chemical mixtures in the environment challenges their in-depth risk assessment due to the diverse list of compounds in use and the lack of toxicity data, as they can induce toxic effects through multiple biological mechanisms. Quantifying the contribution of individual compounds to biological effects requires experimental data to calculate their toxic potency, which is often limited or not available. To address this issue, in silico models can be used to fill in data gaps for compounds with unknown toxic potency. In this study, a quantitative structure-activity relationship (QSAR) model was developed and applied to predict the aryl hydrocarbon receptor (AhR) activity of compounds detected in a monitoring study along the Danube River and to prioritize them for experimental testing. The QSAR model developed in this study was built on the ToxCast data and the prioritized compounds with predicted AhR activity were experimentally validated by the CAFLUX bioassay. The combination of ToxCast data (measured AhR activity) and QSAR model predictions (predicted AhR activity) was used to calculate site-specific AhR mediated activity, which was compared to the overall AhR activity detected in bioassay at sampling sites. Experimental testing confirmed the ability of the QSAR model to identify compounds with high

AhR activity and to prioritize the most relevant suspect effect drivers. Nevertheless, the QSAR model also predicted a high number of AhR inactives as false positives (specifically, as compounds with low AhR activity). Thus, when calculating AhR mediated activity, it is essential to critically assess QSAR prediction results in terms of applicability domain and consider reliability of the predictions according to a defined prioritization scale. The QSAR model contributed to explaining a significant portion of the unexplained AhR activity in the Danube River samples and facilitated the identification of novel environmentally relevant micropollutants accountable for this endpoint. The results also emphasize that despite an extensive list of target compounds and modelling of their activity, part of the AhR effect remains unexplained and the causative compounds unknown. Despite the inherent limitations, framework of this approach has proven to be applicable for priority screening of compounds and quantifying their contribution to detected biological effects in environmental monitoring studies. The work was supported by the European Union's Horizon Europe programme, grant agreement no.101057014 (PARC).

1.07.P-Mo046 Machine Learning Ecotoxicity Assessment of Pharmaceuticals

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In recent years, the massive emergence of new chemical compounds for various uses, such as industrial, medicinal, etc., has been observed, accompanied by the need for ecotoxicological studies and risk analysis on these compounds. To this end, *in vivo* approaches are generally used with living organisms, which raise major ethical concerns and experimental difficulties, such as time and costs. As an alternative, New Approach Methodologies (NAMs), more specifically, *in vitro* and *in silico* approaches, emerge using biological models isolated in controlled environments, and computational models, respectively. Despite still being underexplored, *in silico* approaches have become increasingly relevant as potential alternatives to address several limitations of animal testing methods, taking advantage of the large amount of data already available and of the capacity of specific algorithms to learn from data to increase the potential for predicting ecotoxicity and risk assessment. The present work aims to develop machine learning models to predict the toxicity of pharmaceutical compounds in order to reduce the use of *in vivo* tests, in addition to reducing the time and cost of ecotoxicological analyses. To this end, data were collected from the ADORE database to create models based on the ML algorithms for classifying the acute toxicity (LC50) of pharmaceutical products, using both cheminformatics and biomolecular descriptors as input variables. The preliminary results highlight the importance of the study and the potential of these models to reduce animal testing, serve as valuable auxiliary tools and, in the future, completely replace *in vivo* methodologies when integrated with *in vitro* strategies. Further validation and refinement of these models will be crucial to maximize their accuracy and applicability, paving the way for a more sustainable and ethical scientific approach.

1.07.P-Mo047 The Applicability Domain Concept, A Must for all NAMs: Focus on Machine Learning QSAR models

Floriane Larras, Emel Ay-Albrecht and Paul Thomas, KREATiS, France

New Approach Methodologies (NAMs) play a crucial role in the era of the 3Rs (Replacement, Reduction, and Refinement) to reduce or replace animal testing. Among the numerous approaches encompassed by the term NAMs, *in silico* methods, notably Quantitative Structure-Activity Relationship (QSAR) models stand out from the crowd in terms of their significant potential. These approaches are not only less expensive than experiments but also offer rapid results, often with the added benefit of providing mechanistic insights. To gain confidence in QSAR models and their predictions, criteria were also established by the OECD in 2014 and more fully in 2023 with the advent of the QSAR Assessment Framework (QAF). One of the most important aspects to consider when dealing with prediction reliability is the third OECD principle of the defined applicability domain (AD), which limits the response and chemical space covered by the model. This work is an overview of the existing AD assessment method of QSARs and aims to discuss pros and cons of current approaches. To predict ecotoxicity endpoints, *in silico* models ranging from simple linear regression to more sophisticated models like artificial neural networks can be used. Once the model is developed, there are different ways to characterize the chemical space depending on the chosen modelling approach. These methods are various and present different levels of complexity such as range-based approaches, geometric methods, distance-based approaches, and probability density methods. While these techniques are effective for simpler models, they may lack robustness for more complex applications, as they often focus exclusively on the model chemical space. It is also essential to consider the response space, ensuring that the compound is within the range of chemical space but also that the prediction aligns well with expected outcomes. Advanced AD methods integrate both chemical and response spaces, providing a more comprehensive and accurate model applicability. Numerous advanced techniques address these complexities, enhancing AD accuracy and ensuring that predictions are reliable across diverse scenarios. The examples presented in this presentation

will focus on QSAR approaches. However, since the concept of the AD is relevant to both experimental and computational methods, this work aims to serve as a starting point for broader discussions across all NAMs.

1.07.P-Mo048 Computational Predictions of the Binding Activity of Endocrine Disrupting Compounds to the Estrogen Receptor Alpha: Evaluation of the Applicability of Molecular Docking and Simulations

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Molecular docking, a well-established computational method to aid drug discovery, has found widespread application in endocrinological applications to study the interaction between environmental pollutants and the estrogen receptor alpha (ERα). By screening large chemical spaces and identifying substances of high concern for further testing using in-vivo or in-vitro assays, molecular docking is often used to improve the effectiveness and sustainability of chemical risk assessment. However, the scoring functions used in molecular docking to evaluate binding poses disregard well-known effects associated with ligand binding. Moreover, they provide a coarse approximation of binding affinity while commonly used in-vitro assays report the transcriptional activity induced by ligand binding. This discrepancy necessitates a critical assessment of the capabilities of molecular docking to provide a prediction of binding activity and compare different ligands in a toxicological context. To this end we used AutoDock Vina 1.2.5 to dock known or suspected endocrine disrupting compounds from various classes including alkylphenols, phytoestrogens, parabens, bisphenols and perfluorinated compounds into the ligand binding pocket of the estrogen receptor alpha. Utilizing the relative abundance of structural information on the ERα, we calibrated the docking procedure, with results showing significant improvements in pose prediction when allowing for sidechain flexibility within the binding pocket. The final binding poses predicted through our docking approach show good agreement with known experimental poses, demonstrating the applicability of scoring function to study receptor-pollutant interactions. Additionally, the docking scores determined using VinaXB were compared to available experimental measures from cell-based transactivation assays and cell-free receptor binding measurements, as well to more rigorous relative binding free energy calculations using an alchemical route. However, our results show low correlation between docking scores and receptor activity, advising against the use of molecular docking to rank compounds based on the predicted potency.

1.07.P-Mo049 Integration of In Vitro Mechanism Data from ToxCast/Tox21 Bioassays and In Vivo Toxicity Data into Adverse Outcome Pathway (AOP) Network to Develop Explainable AI Models

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Artificial intelligence (AI) models offer immense potential for evaluating the toxicity of a wide range of environmental chemicals. However, many existing toxicity prediction models function as 'black boxes,' making them difficult for toxicologists to interpret and limiting their acceptance in regulatory contexts. The complexity of mechanisms leading to apical toxicity further complicates the reliability of these models, as results can lack supporting process evidence and, in the worst cases, may be coincidental. The adverse outcome pathway (AOP) framework offers a promising solution by connecting molecular toxicity endpoints to apical outcomes. To address these challenges, this study focused on creating explainable AI models for toxicity prediction using ToxCast/Tox21 data within the AOP framework. The first step involved identifying AOPs relevant to regulatory endpoints. Next, in vitro bioactivity data from ToxCast/Tox21 and in vivo toxicity data aligned with OECD test guidelines from eChemPortaIDB were integrated into AOPs. Machine learning models were then developed to predict toxicity at both mechanistic and regulatory endpoints, achieving strong performance with AUC-ROC values ranging from 0.56 to 0.88. A proof-of-concept case study using reference chemicals further demonstrated that these models effectively screened toxic chemicals with high sensitivity. This research establishes a foundational approach for developing explainable machine learning models using AOPs, addressing a critical limitation in the adoption of AI models for chemical risk assessment. This work was supported by Korea Environmental Industry & Technology Institute (KEITI) through 'Core Technology Development Project for Environmental Diseases Prevention and Management', funded by Korea Ministry of Environment (MOE) (2021003310005).

1.07.P-Mo050 Calibration of Chemical Agnostic Quantitative Adverse Outcome Pathways Based on Multiple Chemical Data using Hierarchical Modelling

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The Adverse Outcome Pathway (AOP) framework facilitates evidence extrapolation from animal and non-animal testing for inferring adverse outcomes, with quantitative AOPs (qAOPs) supporting next-

generation risk assessments. While chemical agnosticity is a fundamental of AOPs, many current qAOP studies faces challenges in accounting for cross-chemical heterogeneity, particularly in response-response relationships where chemical-specific patterns need to be separated from chemical-agnostic pathways. We propose a hierarchical model that explicitly separates chemical-specific from chemical-agnostic patterns in response-response relationships. The method implements chemical-specific parameters sampled from an overarching distribution representing chemical-agnostic patterns, allowing the model to capture varying levels of heterogeneity in multi-chemical datasets. Here, model parameters are inferred in a Bayesian framework, where parameter uncertainty is quantified by the resulting posterior probability distribution. Model evaluation employs Pareto-smoothed leave-one-out cross-validation and stacking for model averaging, with performance assessment through both simulated and real in vitro data from the Risk-Hunt3R project. The proposed calibration approach aims to enhance the generalizability of qAOPs while maintaining their chemical-agnostic nature, potentially supporting more robust next-generation risk assessments. Results from simulation studies showed that our hierarchical model performed better than a model without a hierarchical structure on the response-response relationship, especially when the heterogeneity is high. A case study with real data is presented to demonstrate the model's generalizability in realistic scenarios for derivation of points of departure. The RISK-HUNT3R project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 964537. The opinions expressed in this document reflect only the author's view. The European Commission is not responsible for any use that may be made of the information it contains.

1.07.P-Mo051 The Development and Use of an Adverse Outcome Pathway (AOP)-Informed Cellular Oxidative Stress NAM for Hazard Screening

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Oxidative stress has been described as a prominent mechanism of xenobiotic toxicity and a common denominator in many disease states yet a standalone test does not exist. The conceptual adverse outcome pathway (AOP) framework allows us to organise the key processes involved and monitor them at each level of biological organisation. Thus, a likely series of key events (KE) that leads to oxidative stress and ultimately oxidative cell death can be proposed and tested for. Oxidative stress can occur in any species from bacteria to humans, therefore, as a biomarker it has a wide domain of applicability across species. In this investigation we have used the rainbow trout fish species, *Oncorhynchus mykiss*, as a test model and the RTgill W1 and RTL W1 cells lines to develop such an oxidative stress AOP-informed testing strategy, focusing on monitoring upstream and downstream key events involved in its processes as well as the cells redox status. These events included increases in cellular reactive oxygen species (ROS) levels (KE 1940) and increased lipid peroxidation (KE 1445). The cellular redox status at different exposure times was characterised by monitoring levels of oxidation of glutathione (KE 926). The information from this multi-parametric NAM was integrated and in this way the intensity of an oxidative insult could be characterised into low, intermediate or high intensity. Such an approach can be used to help interpret data from multiple assays, for hazard ranking or indeed to group substances based on their oxidative stress potential. Property-effect relationships can then be established for these different reactivity groups to identify the descriptors that can be linked to such reactivity and used in a predictive toxicology space. This developed NAM was applied when testing the hazard of multi-component mixed metal oxide perovskites. As a benchmark material a CuO nanomaterial was included, for which there is large evidence of oxidative stress association. To provide in vivo anchorage levels of these key events, biomarkers were also assessed in tissues of perovskite exposed fish. Data generated from such cellular based NAMs can be used in weight of evidence (WoE) approaches for hazard assessment and for hazard screening in safe and sustainable by design (SSbD) strategies. HARMLESS grant agreement No. 953183 and General protocol of actions between MITECO and INIA, CSIC for carrying out activities related to substances and chemical mixtures.

1.07.P-Mo052 To What Extent Do Fish Toxicity Studies Drive Acute and Chronic Aquatic Hazard Classification?

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The scheme for classification and labelling of substances and mixtures for aquatic hazards, as defined in Europe under the CLP Regulation (EC) No 1272/2008, relies on identification of the intrinsic hazards posed by chemicals to the environment. Currently, the classification for aquatic hazards focuses solely on

organisms that live in the water column, more precisely on the results of aquatic toxicity tests performed on organisms belonging to the three trophic levels (fish, aquatic invertebrates and algae or other aquatic plants) that are considered as surrogate for all aquatic organisms. Using in vivo fish data for classification purposes raises animal welfare concerns. Therefore, in the light of the ongoing discussions on replacing in vivo vertebrate data within various regulatory frameworks for example through the use of New Approach Methods (NAMs), this study analyses the role and impact of in vivo fish toxicity studies on the harmonised classification and labelling for aquatic hazards in the EU. In this study we have compared the most sensitive study results indicated by the Risk Assessment Committee (RAC) for all three trophic levels per substance, examining whether the substances exhibited similar toxicity across different trophic levels. We investigated whether the same classification would likely have been assigned in the absence of fish data. The study covers formal proposals for Harmonised Classification and Labelling (CLH) and RAC opinions proposing CLH at EU level, published on the ECHA website between July 2009 and December 2023. The final dataset resulted in 281 unique substances whose data led to a classification for aquatic hazards, with data considered scientifically robust and GLP-compliant. The current analysis shows that all three trophic levels play an important role in overall environmental hazard classification. The RAC derived aquatic hazard classification for both acute and chronic hazards was based on fish data (as most sensitive species) in 20% of cases. When comparing the sensitivity difference between three trophic levels, for over half of those cases, the classification would be less stringent if fish data were unavailable. The outcome of this study contributes to ongoing discussions on acute fish IATA and introduction of NAMs. To ensure an adequate level of environmental protection, it is important to investigate how future NAMs could detect, as a minimum, those substances that are most toxic to fish.

1.07.P-Mo053 Development of Acute to Chronic Ratios (ACRs) Applicable to Surfactant Ecotoxicity
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Surfactants find extensive use within a variety of settings, both industrial and domestic. Although typically removed in an efficient manner during the process of wastewater treatment, these substances nevertheless remain liable to enter into the environment. Whilst their safety within such contexts has been widely investigated, the presence of data gaps particularly as relates to chronic toxicity continues to form an obstacle towards meeting regulatory standard information requirements. A potential solution to this issue lies within the leveraging of existing toxicity data, in order to develop acute-chronic ratios (ACRs). In essence, the ACR represents an empirically-derived ratio between corresponding acute and chronic ecotoxicological endpoints (within defined chemical groups), applicable for the estimation of chronic potency in instances where only acute values are otherwise available. Previously, this approach has demonstrated robustness within certain (non-surfactant) substance classes possessing non-specific mechanisms of action. However, surfactants hold several unique structural and physicochemical characteristics. Accordingly, the aim of this investigation was to examine the suitability of ACR methods for application towards such compounds. Details of relevant surfactants, such as Chemical Abstract Service (CAS) number, name and molecular structure (as available), were traced. Using these identifiers, historical, publicly-accessible toxicity data concerning fish, daphnid and algal species were sought, recorded and curated, in order that robust toxicity outcomes acute and chronic might be identified. Several hundred surfactants, spanning anionic, cationic, zwitterionic and non-ionic classes (including mixtures), were ultimately represented. From this pool, ACRs appropriate to each trophic level were determined. Comparison relative to existing ratios revealed that acute-chronic relationships within these chemicals are broadly equivalent to those noted across other narcotic (non-specific acting) substances. As such, evidence for the apparent applicability of the approach within a wider series of risk assessment contexts is provided.

1.07.P-Mo054 Surfactant Endpoint Read-Across Strategies under REACH: A Review of and Guidance for Future Submissions

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One of the common approaches for addressing standard information requirement (SIR) datagaps in registration dossiers under REACH is the use of read-across or grouping argumentation where information from one or a number of analogous (or source) substance(s) is used to support or predict missing endpoint data for a target substance. Methods to support read-across arguments are outlined in the Read Across

Assessment Framework (RAAF) which defines six scenarios. Each scenario is associated with a set of pre-defined Assessment Elements (AEs) which cover the essential scientific aspects needed for that scenario to be acceptable. Most high volume surfactant dossiers were submitted before the RAAF guidance was developed suggesting that it may be possible to develop other acceptable scenarios relevant for surfactants which are not presently listed in the RAAF. Many surfactants grouped into REACH categories share identical/common hydrophilic head-groups, and differ by variations in alkyl chain length/type (linear/branched/unsaturated). Here we provide outputs of a review of current read-across and category/grouping approaches for surfactants focused on the ongoing discussions between ECHA and the relevant REACH consortia, to assess the key scientific limitations covering Human Health and Environmental hazard endpoints. Read across approaches for dossiers which were submitted pre-RAAF were allocated to scenarios synonymous with those defined in the RAAF. The outcome demonstrates a low rate of success of acceptance of the proposed approaches. Key reasons for lack of acceptance included (although not limited to) lack of test item composition, toxicity similarity arguments or data. The aim is to leverage learnings from surfactant dossier compliance check (CCH) decisions and evaluations by ECHA to provide recommendations for appropriate grouping and read-across approaches for surfactant dossiers which have yet to be reviewed.

1.07.P-Mo055 Aquatic Toxicity Profile of Neurotoxic Substances

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The classification hazardous to the aquatic environment under CLP regulation relies on data from fish, and generation of such data raises animal welfare concerns. The Fish Embryo Acute Toxicity (FET) test is a potential alternative to acute fish toxicity study, but neurotoxic substances are considered out of the applicability domain of the method. The aim of this study was to analyse the aquatic toxicity profile of neurotoxic substances to assess whether acute studies on invertebrates would reveal similar toxicity as fish and thus cover the acute aquatic hazards classification of neurotoxicants. Acute data on the aquatic toxicity was collected from Harmonised Classification and Labelling (CLH) reports and ECHA Chemicals Database for 113 substances, which are considered to be neurotoxicants due to the identification of the nervous system as target organ in their harmonised STOT SE and/or STOT RE classifications, or because they induced narcotic effects. The lowest toxicity value of each trophic level was selected to assess which aquatic toxicity test would result in the most stringent classification. If the difference between the lowest toxicity values between the different trophic levels was >10-fold, it was considered that it could lead to difference in classification. Reliable acute toxicity data on fish and invertebrates was available on 50 substances. Comparing the available data on fish and invertebrates, for 26 neurotoxicants the tests with fish resulted in the lowest toxicity value. However, when assessing the impact on the classification, there was a >10-fold difference only for one neurotoxicant. Based on these results, we observed that the acute invertebrate study could mostly cover acute aquatic hazards of substances with neurotoxic properties. For substances where fish was the most sensitive species, the difference between EC/LC50 for fish and invertebrates would not result in a different hazard classification. The findings of this study support the application of FET as an alternative to acute fish studies. For neurotoxic substances falling out of the scope of the FET test, it is likely that the data on invertebrates would result in the same classification. However, this is based on a limited dataset and further research is needed to confirm this outcome. Also, more research is required to determine if FET is suitable for other substances and if it covers acute fish toxicity when fish studies result in the most stringent classification.

1.07.P-Mo056 Use of Read-Across to Fulfil REACH Information Requirements – A Case Study with A Fragrance Ingredient

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The REACH Regulation promotes and supports the 3Rs principles (reduction, refinement, replacement) with obligations to reduce animal use via data sharing, read-across and the implementation of alternative methods. For chemicals manufactured or imported at ? 100 t/y, or already at lower tonnage bands for sparingly water soluble chemicals, information on chronic aquatic toxicity is required, even when safe use can be demonstrated based on PNECs derived from acute aquatic toxicity data, applying high safety factors and con-servative exposure scenarios. If new fish chronic data are needed, a Fish Early-life Stage Toxicity Test according to OECD 210 is the default option. The number of fish used in these studies is approximately 700-850 per study. In this poster, we present how read-across from dodecanal and 2,6,10-trimethylundec-9-enal (source substances) to 2-methylundecanal (target substance) has been used for the

REACH registration to replace the need for such an in vivo study. The case study demonstrates: Practical challenges in conducting the daphnia and fish chronic studies for the two difficult-to-test source substances (volatile, low water solubility, degradation of test item due to oxidation, presence of degradation products, etc.). Comparison of physico-chemical properties and environmental endpoints. Read-across justification based on similar carbon chain length and same mode of action (e.g. ECOSAR class, mono aldehyde) for target substance and source substances. Impact of new data generation on Predicted No Effect concentrations (PNEC), the Chemical Safety Report (CSR) and environmental classification and labelling of source and target substances. Based on the above, conducting an OECD 210 study on the target substance was not considered necessary, and was therefore waived. The application of animal-alternative methods to the above REACH registered substance covered by the above case study save in excess of 800 fish, and demonstrates that alternative, yet conservative, evaluation approaches can be adopted to fill higher-tier hazard end-points without compromising safe use.

1.07.P-Mo057 Application of NAMs and Refinement Strategies in the Petrochemical Industry

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To comply with global regulations and properly assess environmental risk, petrochemical companies develop hazard information for products, substances, and wastewater effluents. New approach methodologies (NAMs), including in silico and in vitro tools, together with refinement strategies for existing in vivo tests, are being explored to generate this hazard data while limiting animal testing. This poster demonstrates the different areas where NAMs and refinement strategies are being used in the petrochemical sector and the challenges associated with their application. In silico tools (including QSARs; e.g., EPISuite or PETROTOX), prioritization approaches and read-across strategies (e.g., hydrocarbon block methodologies) are often used to generate petrochemical hazard data while avoiding animal testing. However, the potential applicability of in vitro NAMs for petrochemical hazard data generation is still being assessed. Current projects include exploring the applicability of the OECD 249 fish cell line test to predict the toxicity of consumer-use petrochemicals (solvents, surfactants), and the use of the OECD 321 HYBIT test to assess petrochemical bioconcentration. These projects have highlighted challenges associated with the application of NAMs for petrochemical substances. For example, use of in silico tools can be limited for some petrochemical substances which display physico-chemical and structural characteristics outside of model applicability domains. Similarly, petrochemical substances tend to be associated with difficult test properties, which introduce technical issues when designing and interpreting output from in-vitro tests. Given that in vivo fish data are still required for registration of high-tonnage substances regulated under EU REACH and for off-shore chemicals governed through the Oslo-Paris Convention (OSPAR), the refinement of current in vivo testing protocols and alignment of current regulatory practices is also being evaluated along with the use of the OECD 236 FET test for petrochemical testing. Notably, the lack of alignment of chemical regulations, e.g. OSPAR and REACH, can result in a duplication of animal testing for petrochemicals. Altogether, this poster highlights the efforts and challenges associated with the application of NAMs in the petrochemical industry and the importance of continuous exploration of their use to ensure the safety of the products and wastewater effluents while reducing animal testing.

1.08 In silico New Approach Methodologies for Enhancing Understanding of Chemical Safety: Getting your Questions Answered by the Developers

1.08.T-01 The Exposure And Safety Estimation (EAS-E) Suite Platform to Aid Chemical Assessments

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Environmental and human health risk assessments require quantitative exposure data for thousands of chemicals in various systems. Understanding the processes that determine chemical exposure provides critical insights for risk management decisions for the safe and sustainable use of chemicals by society. Multi-media mass-balance fate, toxicokinetic (bioaccumulation), and exposure models are often necessary for exposure and risk estimation. These mechanistic (process-driven) models require chemical-specific input data such as physical-chemical properties, toxicokinetic data, environmental degradation half-lives, production volumes and emission rates. However, for many of the chemicals requiring evaluation, these data are limited, not standardized and documented across multiple databases. To reduce animal use and address significant data gaps, New Approach Methods (NAM) like in vitro bioassays and validated Quantitative Structure Activity Relationships (QSARs) can be used; however, their application often

requires expert judgement and a careful evaluation of the results (e.g. Applicability Domain). Most importantly databases and models are not integrated; chemical evaluators need to gather information from many diverse sources to perform assessments. Here we introduce the Exposure And Safety Estimation (EAS-E) Suite platform to bridge the gap between evolving scientific research and regulatory assessment challenges. EAS-E Suite is a free on-line platform developed to facilitate knowledge translation to various stakeholders on issues in exposure assessment and environmental health. EAS-E Suite is comprised of chemical information databases, QSARs for predicting chemical information if measured data are unavailable, and models and tools to aid chemical assessment decision-making. EAS-E Suite provides opportunities to address regulatory challenges for new and existing chemical assessments for ecological and human health objectives to support a One Health approach to chemicals management and to guide the safe and sustainable production and use of chemicals in society. A case study illustrates how the platform integrates various data sources and knowledge across multiple disciplines to reduce uncertainty, improve chemical management and support decision-making.

1.08.T-02 Web-Based Platforms for Toxicogenomics Data Analysis: EcoToxXplorer and ExpressAnalyst

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Toxicogenomics data are likely to play a key role in the transition from traditional to alternative toxicity testing methods. The costs of acquiring such data continue to drop, and an ever-increasing number of researchers and regulators would like to get involved in the world of toxicogenomics. However, these data are complex, and analyses typically require advanced programming skills and a deep knowledge of statistics and genomics resources, and as such, are usually handled by expert bioinformaticians. The objective of this presentation is to outline two freely accessible web-based tools that we have been developing: ExpressAnalyst (www.ExpressAnalyst.ca) and EcoToxXplorer (www.ecotoxxplorer.ca). ExpressAnalyst has been designed to process, analyze, and interpret RNA-sequencing data from any eukaryotic species. EcoToxXplorer has been designed to analyze qPCR data measured with custom EcoToxChip arrays from six ecological species (model organisms: Japanese quail, fathead minnow, African clawed frog; native species: double-crested cormorant, rainbow trout, northern leopard frog). Both are viewed as next-generation bioinformatics tools that are high performance, intuitive, and universally accessible to handle transcriptomics data for the purpose of chemical risk assessment and environmental management. The pipeline for each includes steps for QA/QC, filtering and normalization, differential analysis, interactive functional analysis, and report generation. Both contain FAQs, tutorials, and case studies to aid the user. The functional analysis is toxicology-focused, including integration with the AOPwiki and our custom EcoToxModule gene sets that were designed for high-level interpretation of toxicogenomics data (for EcoToxXplorer), and in ExpressAnalyst users can generate transcriptomic points of departure (tPOD) values. The development of these tools was organized around design-thinking principles in that we iteratively presented the tool to various user groups from academia, government, and industry for testing, and then refined it based on their feedback. These tools were developed through funding from several Genome Canada grants.

1.08.T-03 EnviroTox: A Curated Aquatic Toxicology Database

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Chemical safety assessments require innovative approaches to predict ecological hazards efficiently while reducing reliance on animal testing. One such method is the ecological threshold for toxicological concern (ecoTTC). The underlying data are crucial for a robust ecoTTC computation, but existing individual databases were not directly suitable to support ecoTTC development. This presentation will focus on the EnviroTox database, which was developed to provide a robust, curated database containing high-quality aquatic toxicity studies traceable to the original information source that can be leveraged to support the development of New Approach Methodologies (NAMs) such as ecoTTC. Version 2.0.0 of the EnviroTox database contains 80,912 records representing 1,641 species and 4,267 unique chemical CAS numbers. Data was sourced from 11 different data sources, including the US EPA ECOTOX knowledgebase, ECHA REACH registration information, and the OECD QSAR toolbox. Stepwise Information-Filtering Tool (SIFT) criteria were implemented to identify which data should be included in the EnviroTox database to ensure relevance and quality. The data includes relevant identifiers, physical-chemical properties,

taxonomic information, MOA classifications, and toxicity endpoint information. All entries are traceable to their original source. A search interface allows users to search the database by substance, taxonomy, test, and/or substance properties. In addition to the database, additional tools allow for the generation of regional Predicted No Effect Concentrations (PNECs), ecoTTCs, and Chemical Toxicity Distributions (CTDs). EnviroTox exemplifies how well-curated databases can aid in chemical safety assessments. While this database was initially developed to calculate ecoTTC and CTD values, it provides a unique resource due to the many data sources and the curation of the datasets. It has been leveraged for many project types. A search of publications citing the database in 2024 yields manuscripts that leveraged the database for QSAR development, creation of species sensitivity distributions⁶, machine learning-based predictions, and other uses, demonstrating the broad range of applications for the database and the included tools.

1.08.T-04 Leveraging Molecular Docking Techniques to Support Virtual Screening of Cross-species Susceptibility to Chemical Effects

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Understanding cross-species susceptibility to chemical exposures poses challenges in toxicology and environmental risk assessment. Traditional experimental methods to evaluate these effects are resource-intensive and impractical for high-throughput screening. A significant knowledge gap exists in how variations in protein structures influence chemical binding and subsequent biological effects. Bridging this gap is essential to augment predictions of chemical sensitivities across species and support environmental risk assessment and biodiversity protection. This study presents a computational framework that leverages the availability of genomic data, advancements in ortholog prediction tools, and AI-based protein folding technologies to facilitate accurate prediction of molecular initiating events (MIEs) within the Adverse Outcome Pathway (AOP) framework. These advancements enable the integration of molecular docking to unravel mechanisms underlying species-specific susceptibility to chemical agents. As a case study, we applied this approach to acetylcholinesterase (AChE), a key enzyme involved in neurotransmission across various taxa. Homology modelling was used to generate AChE ortholog protein structures from Lepidopteran species, followed by molecular docking to evaluate interactions and binding affinities with chlorpyrifos and its active metabolite, chlorpyrifos oxon. The framework also aimed to predict off-target effects through flexible docking and evaluate ligand interactions across diverse protein targets. Molecular simulations were used to assess the stability and conformational dynamics of protein-ligand complexes, providing deeper insights into the kinetics of binding. The analysis added biological context by identifying unintended interactions and mapping enzymes underpinning phase I metabolism, such as cytochrome P450-mediated detoxification, thereby elucidating species-specific chemical processing pathways. This approach addresses critical gaps in understanding protein chemical interactions by incorporating advanced protein structure prediction techniques and molecular docking simulations to refine predictions of species-specific chemical impacts. It contributes an additional line of evidence within New Approach Methodologies (NAMs) and supports Next-Generation Risk Assessment (NGRA), enhancing the accuracy of ecological risk assessments and informing regulatory decisions to safeguard ecosystems and public health.

1.08.P-Tu050 QSAR-ME Profiler 2025: QSAR Predictions, Similarity Analysis, Domain Inspection and Metabolites Profiling

Nicola Chirico, Marco Evangelista and Ester Papa, QSAR Research Unit in Environmental Chemistry and Ecotoxicology, Department of Theoretical and Applied Sciences, University of Insubria, Italy

In the context of environmental risk assessment, *in silico* tools play a pivotal role in the prediction of chemical activity. Among these tools, Quantitative Structure/Property Activity Relationships, abbreviated as QSA(P)Rs, are well-known alternatives to *in vivo* / *in vitro* experiments. In the last 20 years, more than 100 QSA(P)Rs were developed by the QSAR research unit of the University of Insubria, targeting physical-chemical properties, global indexes, ecotoxicology and metabolic transformations. To streamline the application of these QSA(P)Rs, and to simplify the evaluation of their predictions, a new multiplatform software called QSAR-ME Profiler 2025 has been developed, which improves and restyles the previous version published at <http://dunant.dista.uninsubria.it/qsar/>. This new version continues to detail QSA(P)Rs data and corresponding predictions, to check the applicability domain compliance of the QSA(P)Rs, and to allow for automatic detection of the most similar chemicals, to further aid assessment of the chemicals under scrutiny. However, the new version now also provides the possibility to transform endpoints of MLR QSA(P)Rs to better suit regulatory purposes. In addition, the new version now provides uncertainties of the predictions by the classification models and defines the applicability domain in

classification which can be checked for new chemicals of interest. Combined predictions can still be calculated when multiple QSA(P)Rs are available for a certain endpoint. Like the previous version of the QSAR-ME Profiler, Toxtree software with the SMARTCyp module is used to automatically select QSARs for the prediction of in vitro biotransformation, that were developed according to the cytochrome P450-mediated drug metabolism predicted by SMARTCyp. The new version of the QSAR-ME Profiler also allows for the identification of the metabolites of the chemicals under scrutiny, detected by SMART Cyp, which can be profiled using multiple QSARs. This new option allows for better screening of the potential total hazard of the studied chemicals and their metabolites. QSAR-ME Profiler 2025 is available free of charge at <http://dunant.dista.uninsubria.it/qsar/>.

1.08.P-Tu052 Computational Approach to Evaluate Cross-Species Binding Affinity and Susceptibility of Endocrine Disrupting Chemicals in Consumer Products

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Endocrine Disrupting Chemicals (EDCs) have been widespread in consumer products, potentially impacting human health and ecosystems by targeting androgen (AR) and estrogen receptors (ER). Currently, data on the impacts of most consumer product chemicals on non-target species are limited or lacking. This study aims to assess cross-species susceptibility to selected EDCs and chemicals in cleaning products by identifying species with high orthologous similarity to human AR and ER and comparing binding affinities across humans and other species. To achieve these goals, we employed Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) to identify species with receptor structures orthologous to human AR and ER, verified using AlphaFold modeling. Molecular docking was then performed on chemicals present in cleaning products, as well as on other high-toxicity chemicals, to analyze their binding affinities with identified orthologous receptors across 9 species (fish, birds, and endangered species). Chemicals frequently found in consumer products, such as Bisphenol A, triclocarban, and triclosan, demonstrated high binding affinity to the Estrogen Receptor (ER). SeqAPASS analysis identified 800 orthologous species for ER, enabling predictions of binding affinities and corresponding susceptibilities across these species. This analysis encompassed all species included in the molecular docking queries, highlighting the interconnectedness between protein similarity and binding affinity. However, this study integrates orthologous alignment and molecular docking approaches to offer a cross-species susceptibility assessment, identifying species particularly sensitive to chemical exposure in consumer products. Insights derived from this computational approach could inform future chemical safety assessments by enhancing predictions of species-specific susceptibility to EDCs across human and non-human species. This work was supported by Korea Environment Industry & Technology Institute (KEITI) through Technology Development Project for Safety Management of Household Chemical Products, funded by Korea Ministry of Environment (MOE)(RS-2023-00215309)

1.08.P-Tu053 An Open-Source Computational Pipeline for Predicting Binding Affinity and use in Chemical Hazard and Species Sensitivity Ranking

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A challenge in environmental and ecological risk assessment is to identify which chemicals are of most concern and which species are most vulnerable particularly for endocrine disrupting chemicals. A mechanism of endocrine disruption is the binding of chemicals to the steroid receptor outcompeting the natural ligand to act as either agonists or antagonists. Synthetic glucocorticoids are steroids used extensively to treat a variety of health conditions and are a growing environmental concern in the aquatic environment. The current study presents a computational pipeline using open-source software to predict the binding affinity of 12 synthetic glucocorticoids, 5 other chemicals that have been reported to interfere with glucocorticoid receptor functioning and the natural ligand cortisol to 163 teleost fish glucocorticoid receptors. It is those chemicals with the highest binding affinity that will out compete cortisol at the receptor binding site and pose the greatest hazard. In this exercise the glucocorticoid halcinonide was predicted to be the most hazardous chemical based on the predict binding affinity and species from the superorder Protacanthopterygii, containing the Esociformes and Salmonidforms as the most vulnerable. This pipeline can be extended to more chemicals, species and other proteins as an in silico chemical hazard assessment too and for species sensitivity ranking based on interference at the molecular initiating event of an adverse outcome pathway. Funding source - UKRI Natural Environmental Research Council grant reference NE/X000192/1

1.08.P In silico New Approach Methodologies for Enhancing Understanding of Chemical Safety: Getting your Questions Answered by the Developers

1.08.P-Tu048 The Many Pros and a Few Cons of Mechanistic In Silico NAMs

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It is critical that NAMs rapidly obtain the same recognition and support by regulatory authorities as empirical methods if the Green Deal is ever to attain the speed and precision necessary to meet the demands of eco-design. Mechanistic QSARs (i.e. any quantitative relationships observed between structures and effects on biological organisms) can help us to the necessary paradigm shift. But we must define Mechanistic in the context of in silico (eco)toxicology. In the context of in silico expert-knowledge-based models (Q)SARs, mechanisms of toxic action (MechoA) are inextricably linked to two keystones: on one side structural features and on the other biological matrix interactions. Unlike read-across methods, more than one structural group can have the same mechanism of action (thus, the same impact at the same molar concentration). Mechanistic insight (i.e., Structural Alerts or SARs) is a necessary first step in producing a reliable QSAR with a well-defined applicability. MechoA was developed to do this rapidly. It provides an understanding of the interactions between the test substance and the biological matrix that can explain the observed toxicity and link it to structurally similar (or different) chemicals. Any endpoint can ultimately be related to a MechoA (SAR) but the QSAR produced will be related to the species, biology, timeframe of the study, compartment of concern and apical endpoint of interest (e.g. chronic toxicity to fish following OECD 210 Guideline). Moreover, working on the MechoA (merging structures within a same QMAR) allows the development of more robust models with greater chemical space coverage rather than being limited to one structural group. Furthermore mechanistically understanding limits the need for a validation set (for the regulatory purposes). Limitations: Mechanisms only work when we know them. Thus, it is useful to use complementary NAMs (e.g. in vitro) to validate outcomes. some datapoints cannot fall under OECD 5 Principles (poor goodness of fit for flat lines; no unambiguous algorithm if toxicity > solubility). To conclude, Mechanistic NAMs not only add a new arm to the battery of modern alternatives but they add additional advantages that rarely exist in empirical tests. Not only can they replace an experimental endpoint but they can explain it, fit it within the context of an Adverse Outcome Pathway and indicate the potential for more or less insidious toxic mechanisms that need supplementary attention.

1.08.P-Tu049 Performance Evaluation of QSAR Model “KATE2020” in Algal Toxicity Prediction using CSCL Newly Announced Chemical Substances as Validation Set

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As there is no or little hazard information on most of the organic chemicals currently in the market, expectations have been growing for the use of alternative methods to toxicity testing such as quantitative structure-activity relationships (QSAR). National Institute for Environmental Studies has been developing QSAR model “Kashinhou Tool for Ecotoxicity (KATE)”, which predicts acute and chronic toxicity of industrial organic chemicals to aquatic organisms by classification based on chemical structure and linear regression using octanol/water partition coefficient as a descriptor, and judges applicability domain (AD) regarding structure and descriptor. In this presentation, we will report on the performance evaluation results of KATE2020 ver. 5.1 in the algal toxicity prediction using newly announced chemical substances under Chemical Substances Control Law of Japan (CSCL new chemicals). Measured acute and chronic toxicity data on CSCL new chemicals to algae were collected from the web site (www.nite.go.jp/chem/jcheck/list7.action?category=240). In addition, data on OECD high production volume chemicals (OECD HPV chemicals) were also used as a comparison because CSCL new chemicals can be structurally more complex, and the predictive performance can be different between the datasets accordingly. Toxicity values of chemicals in the datasets were predicted using KATE2020 and US EPA's ECOSAR2.2 model that predicts ecotoxicity by classification and regression, which is similar methods to KATE, and (i) percentage of chemicals predicted within AD, (ii) for those within AD, percentage of predicted toxicity values that fall within a factor of 10 in ratio to measured toxicity values were computed. (i) 45% and 57% of CSCL new chemicals were predicted within AD by KATE2020 for acute and chronic toxicity, respectively. (ii) For predictions within AD, 80% and 79% of those in KATE2020 fell within a factor of 10 in ratio to measured toxicity values for acute and chronic, respectively. In comparison to ECOSAR2.2, KATE2020 predicted less chemicals within AD but obtained higher prediction accuracy for chemicals judged within AD. This would be because KATE2020 judges AD regarding structure and descriptor to ensure interpolation, even though predictions within AD are not necessarily used as alternative to toxicity testing. It is expected that including CSCL new chemicals in the training set of KATE2020 will expand its AD.

1.08.P-Tu050 QSAR-ME Profiler 2025: QSAR Predictions, Similarity Analysis, Domain Inspection and Metabolites Profiling

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In the context of environmental risk assessment, *in silico* tools play a pivotal role in the prediction of chemical activity. Among these tools, Quantitative Structure/Property Activity Relationships, abbreviated as QSA(P)Rs, are well-known alternatives to *in vivo* / *in vitro* experiments. In the last 20 years, more than 100 QSA(P)Rs were developed by the QSAR research unit of the University of Insubria, targeting physical-chemical properties, global indexes, ecotoxicology and metabolic transformations. To streamline the application of these QSA(P)Rs, and to simplify the evaluation of their predictions, a new multiplatform software called QSAR-ME Profiler 2025 has been developed, which improves and restyles the previous version published at <http://dunant.dista.uninsubria.it/qsar/>. This new version continues to detail QSA(P)Rs data and corresponding predictions, to check the applicability domain compliance of the QSA(P)Rs, and to allow for automatic detection of the most similar chemicals, to further aid assessment of the chemicals under scrutiny. However, the new version now also provides the possibility to transform endpoints of MLR QSA(P)Rs to better suit regulatory purposes. In addition, the new version now provides uncertainties of the predictions by the classification models and defines the applicability domain in classification which can be checked for new chemicals of interest. Combined predictions can still be calculated when multiple QSA(P)Rs are available for a certain endpoint. Like the previous version of the QSAR-ME Profiler, Toxtree software with the SMARTCyp module is used to automatically select QSARs for the prediction of *in vitro* biotransformation, that were developed according to the cytochrome P450-mediated drug metabolism predicted by SMARTCyp. The new version of the QSAR-ME Profiler also allows for the identification of the metabolites of the chemicals under scrutiny, detected by SMART Cyp, which can be profiled using multiple QSARs. This new option allows for better screening of the potential total hazard of the studied chemicals and their metabolites. QSAR-ME Profiler 2025 is available free of charge at <http://dunant.dista.uninsubria.it/qsar/>.

1.08.P-Tu051 Predictive Model for Identifying Thyroid Endocrine Disrupting Chemicals Using the Comptox Chemicals Dataset

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Thyroid hormones are essential to the endocrine system, and their disruption by chemicals is a major toxicological concern. Traditional methods, such as animal experiments and epidemiological studies, are limited in scope and not efficient for evaluating the vast number of novel chemicals. To efficiently screen the toxicity of a large number of chemicals, it is more effective to first predict toxicity using existing data and then validate the results with an alternative animal toxicity test model, such as zebrafish, rather than relying solely on traditional methods. In this study, we developed a deep learning model to classify chemicals based on their potential to disrupt thyroid hormone activity. Chemical structures were encoded as SMILES and then converted into 2048-bit Morgan fingerprints using RDKit. The deep learning framework was designed as a hybrid architecture employing a multi-branch structure. Convolutional Neural Networks (CNN) and Long Short-Term Memory (LSTM) networks were integrated to extract relevant features, and the Dense Layer and Attention mechanism combined and improved the features for the final prediction. This approach leverages the strengths of different neural network architectures to effectively capture important patterns in chemical fingerprints for binary classification. A sigmoid activation function was applied to the output layer, and Focal Loss was used to address class imbalance. The final model consistently achieved an F1 score exceeding 0.8 in most test cases, indicating its robust and reliable predictive capabilities. To validate the model, zebrafish embryos were exposed to sub-lethal concentrations of selected chemicals for 96 h and changes in gene expression were measured. This study demonstrates the potential of computational models to efficiently screen large chemical datasets for endocrine-disrupting chemicals. Future studies are needed to expand the scope of model to other hormone-related pathways and enhance its predictive accuracy with additional chemical descriptors. This study was supported by the National Research Foundation of Korea (NRF; Project no. RS-2023-00251751)

1.08.P-Tu052 Computational Approach to Evaluate Cross-Species Binding Affinity and Susceptibility of Endocrine Disrupting Chemicals in Consumer Products

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Endocrine Disrupting Chemicals (EDCs) have been widespread in consumer products, potentially impacting human health and ecosystems by targeting androgen (AR) and estrogen receptors (ER).

Currently, data on the impacts of most consumer product chemicals on non-target species are limited or lacking. This study aims to assess cross-species susceptibility to selected EDCs and chemicals in cleaning products by identifying species with high orthologous similarity to human AR and ER and comparing binding affinities across humans and other species. To achieve these goals, we employed Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) to identify species with receptor structures orthologous to human AR and ER, verified using AlphaFold modeling. Molecular docking was then performed on chemicals present in cleaning products, as well as on other high-toxicity chemicals, to analyze their binding affinities with identified orthologous receptors across 9 species (fish, birds, and endangered species). Chemicals frequently found in consumer products, such as Bisphenol A, triclocarban, and triclosan, demonstrated high binding affinity to the Estrogen Receptor (ER). SeqAPASS analysis identified 800 orthologous species for ER, enabling predictions of binding affinities and corresponding susceptibilities across these species. This analysis encompassed all species included in the molecular docking queries, highlighting the interconnectedness between protein similarity and binding affinity. However, this study integrates orthologous alignment and molecular docking approaches to offer a cross-species susceptibility assessment, identifying species particularly sensitive to chemical exposure in consumer products. Insights derived from this computational approach could inform future chemical safety assessments by enhancing predictions of species-specific susceptibility to EDCs across human and non-human species. This work was supported by Korea Environment Industry & Technology Institute (KEITI) through Technology Development Project for Safety Management of Household Chemical Products, funded by Korea Ministry of Environment (MOE)(RS-2023-00215309)

1.08.P-Tu053 An Open-Source Computational Pipeline for Predicting Binding Affinity and Use in Chemical Hazard and Species Sensitivity Ranking

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A challenge in environmental and ecological risk assessment is to identify which chemicals are of most concern and which species are most vulnerable particularly for endocrine disrupting chemicals. A mechanism of endocrine disruption is the binding of chemicals to the steroid receptor outcompeting the natural ligand to act as either agonists or antagonists. Synthetic glucocorticoids are steroids used extensively to treat a variety of health conditions and are a growing environmental concern in the aquatic environment. The current study presents a computational pipeline using open-source software to predict the binding affinity of 12 synthetic glucocorticoids, 5 other chemicals that have been reported to interfere with glucocorticoid receptor functioning and the natural ligand cortisol to 163 teleost fish glucocorticoid receptors. It is those chemicals with the highest binding affinity that will out compete cortisol at the receptor binding site and pose the greatest hazard. In this exercise the glucocorticoid halcinonide was predicted to be the most hazardous chemical based on the predict binding affinity and species from the superorder Protacanthopterygii, containing the Esociformes and Salmonidforms as the most vulnerable. This pipeline can be extended to more chemicals, species and other proteins as an in silico chemical hazard assessment too and for species sensitivity ranking based on interference at the molecular initiating event of an adverse outcome pathway. UKRI Natural Environmental Research Council grant reference NE/X000192/1

1.08.P-Tu055 Evidence-Based Model-Informed Platform to Integrate Knowledge and Data Towards Prediction of Apical Toxicities

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Traditional animal testing in pesticide and drug development faces increasing scrutiny due to ethical concerns and limitations in predicting human outcomes. This study presents an innovative framework integrating in silico New Approach Methodologies (NAMs) through two case studies to predict apical toxicity about ecological and human health risk assessment.

The first case study combined PBPK modeling with QSAR methods to predict bobwhite quail toxicity. The methodology involved careful curation of the ECOTOX database, considering parameters like observation duration and compound purity. The PBPK model incorporated cross-species extrapolation of ADME parameters from humans, chickens, and rats, scaled for quail physiology. The integrated approach achieved exceptional accuracy ($R^2=0.92$, $Q^2=0.83$) across 33 compounds for QSAR analysis and 12 compounds for PBPK modeling with prediction capability about mortality for untested prolonged exposure of new compounds. The second case study investigated apical lipotoxicity caused by chemical-induced inhibition to efflux of lipids through systems modeling of lipid metabolism, focusing on gender-specific responses in liver and small intestine. The model incorporated omics data from preclinical species, and pharmacology review data from NDA reports and utilized SeqAPASS for cross-species

extrapolation. The results demonstrated successful prediction of more severe lipotoxicity in female rats and accurate modeling of organ-specific responses across nonrodent species. The framework represents a significant advancement in toxicological assessment by creating a unified platform that reduces animal testing while prediction point of departure on apical level. The modular design ensures transparency and adaptability, allowing seamless integration of emerging technologies and high-throughput data. This approach aligns with regulatory requirements while offering several advantages: (i) reduced animal testing and associated costs, (ii) improved prediction accuracy through integrated data analysis (iii) enhanced understanding of species/gender-specific responses (iv) flexible adaptation to emerging technologies. This modeling framework demonstrates the potential for NAMs to transform toxicological assessment while addressing ethical concerns and improving scientific outcomes.

1.08.P-Tu056 Let's FOCUS: Temperature in Population Models

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Effect models like the Dynamic Energy Budget (DEB) and the General Unified Threshold Model of Survival (GUTS), when integrated with Individual-Based Models (IBM), provide a tool for higher tier ERA. This study enhances a previously published DEB-IBM framework to improve realism and applicability for case-study scenarios. Key advancements include: (1) evaluating temperature correction mechanisms using experimental data, (2) incorporating feedback mechanisms such as surface-to-volume scaling and growth dilution into the GUTS module, and (3) applying a dynamic temperature scenario derived from the D3 FOCUS profile. Experimental datasets of *Gammarus pulex* exposed to imidacloprid and flupyradifurone at four temperatures (7°C, 11°C, 15°C, and 18°C) were analyzed. Individual datasets were fitted separately to assess temperature-dependent parameter behavior, and subsequently used to fit a temperature-explicit GUTS-RED-T. Results revealed a negative temperature dependence for k_d (dominant rate constant) and m_w (threshold value). These trends were incorporated into the DEB-GUTS-T model. Two population model versions DEB-GUTS and DEB-GUTS-T were simulated under two different dynamic temperature scenarios (i.e., reference and future climate). Simulations showed reduced dose-response relationships in DEB-GUTS-T compared to DEB-GUTS. However, the observed laboratory data indicated increased toxicity with higher temperatures, underscoring discrepancies between population model predictions and experimental findings. These results highlight the complexity of temperature effects on toxicity and emphasize the need to refine temperature corrections in DEB and GUTS modules when used combined in a population model. Overall, this work advances our understanding of DEB-IBM frameworks, facilitating their potential for use in higher-tier environmental risk assessments.

1.08.P-Tu057 Initial Experience with the Application of In Silico Methods in the Context of the New EFSA/ECHA Guidance on the Impact of Water Treatment Processes on the Production of Drinking Water

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The impact of water treatment processes on residues or metabolites of active substances used in plant protection or biocidal products has recently become a major topic in the EU. Starting from April 1st 2026, applicants for approval of actives substances used in plant protection or biocidal products and applicants for authorisation of respective products in the EU need to comply with the new EFSA/ECHA guidance on the impact of water treatment processes for the production of drinking water (EFSA Journal 2023;21(8), 1-108). Within a tiered framework, the new guidance implements the use of in silico modelling techniques to assess transformation products (TPs) that may be formed during disinfection treatment processes. The guidance stipulates a parallel assessment of predicted TPs provided by in silico models and existing literature data. A prioritised list for the predicted TP is to be created based on the likelihood of their production and the documented detection in the water. The overall aim is to determine which kind of functional groups could be formed due to various treatment processes. The outcome of this exercise will be used to support suspect screening analysis in the follow up experimental step and possible hazard assessment, to avoid challenging, expensive and unnecessary testing, especially vertebrate testing. In this poster, we will present initial experiences - but also address limitations when using a battery of in silico tools as proposed in the guidance, to assess the impact of drinking water treatment.

1.09.A Impact of Micro- and Nanoplastics on Environmental and Human Health: Monitoring, Fate, Exposure, Toxicity and Mechanisms of Toxic Action

1.09.A.T-01 Microplastics in Irrigation Water: Spatiotemporal Changes Reveal Their Source and Distribution Characteristics

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This study investigates microplastic pollution in Taiwan's Taoyuan irrigation system, emphasizing spatial-temporal distribution, polymer composition, sources, and ecological risks. Six sampling sites (IC1-IC6), representing agricultural, residential, and industrial areas, were strategically selected across the irrigation zone, which sources water from Shihmen Reservoir, storage ponds, and river weirs, flowing through Pingzhen, Zhongli, Yangmei, and Xinwu districts. Field sampling was conducted monthly from November 2019 to October 2020, with approximately 5 L of surface water collected per site using a stainless-steel sampler, accompanied by three replicate samples per location. Samples were treated with 30% hydrogen peroxide (H₂O₂) at 65°C to remove organic materials and pretreated with ACS-grade sodium chloride (NaCl) to ensure microplastics remained suspended. The suspended particles were filtered (0.45 µm), examined microscopically, and categorized by shape (fiber, film, fragment, foam) and color (white/transparent, black, multicolor). Microplastics (20-5000 µm) were analyzed using micro-Fourier transform infrared (µ-FTIR) spectroscopy with ATR mode, and polymer types were verified with 70% matching accuracy against a spectral library. The findings revealed that microplastics were unevenly distributed, with higher concentrations near densely populated areas and urban runoff sites. Seasonal variations showed peak concentrations during rainy seasons and typhoon events, with the highest abundance recorded in May, ranging from 1.88 to 141 items/L. Predominant polymers included polypropylene, polyethylene, and polystyrene, with fibers (36.64%) being the most common shape. Microplastics were primarily 333-1000 µm in size and white/transparent in color. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) identified population density, lateral canals, and climatic factors as key influences on microplastic distribution. Risk assessments using the polymer risk index indicated significant ecological risks throughout the year, except for the lower risks observed in November and January. This study establishes a baseline database on microplastic pollution in Taiwan's irrigation systems, highlighting the land-based contribution of microplastics and supporting the development of strategies for environmental sustainability.

1.09.A.T-02 Evaluation of Toxicological Characteristics of Airborne Nano/Microplastic Particles From the Industrial Environment

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Micro and nano plastic (MNPs) particles and fibres represent a global scale contamination source with hazard potential. They can be identified all around our environment, even in air, soil and water. One of the major global MNPs polluters is the textile industry, which generates plastic particles (nylon, polyester, polyurethane, polyolefin, acrylic etc.) during the manufacturing processes. The study analyses the level of particle concentration outside a company that processes textile yarns, but also from the proximity of workplaces using an online air samplers with cascade and mono filter holders for silicon (Si) and quartz filters, respectively. A laser detector was used for real-time counting of particles of six size classes (PM_{0.3}-PM₁₀). Silicon (Si) filters with a diameter of 9 mm and pore sizes of 10 µm and 1 µm and quartz filters with a diameter of 25 mm and a pore size of 1.2 µm were used for particle sampling. A special holder was designed and made for selective sampling (PM₁₀ and PM_{1.0}) on Si filters. Quartz filters were pre-calcined (800°C) before collection. Sampling parameters: airflow (GilAir Plus pump) -2 l/m³, collection time -3 hours; starting time 7.00 am (T₁)/2.00 pm (T₂), intermittent natural ventilation, average temperature and humidity: 23.3 °C, RH: 52.5%. The weight of particles collected (µg) on the quartz filters was 4.9E-05 (T₁) and 5.1E-05 (T₂) and on the Si filters 3.2 E-05(T₁) and 5E-06(T₂). The total number of the outside particles (PM_{0.3}-PM₁₀) was situated between 3584,58 (T₁)-15165,05(T₂) and inside 9901(T₁) and 12921,61(T₂). The highest number of PM₁₀ particles inside was 72/m³ and of PM_{0.3} particles 71979/m³. The samples were characterized using electron microscopy and µRaman spectroscopy. All samples showed a high concentration of particles on the filter. Samples show only particles with irregular shapes. The equivalent particle diameter was for PM₁₀: 1-5 µm and PM₁<1 µm. The total Ion Chromatogram shows the presence of PET-type polymer and the TED GS-MS analysis

shows that the weight of the collected particles is PET. After extracting the particles from the quartz filters, they were tested for evaluation of the cytotoxic potential of preparations on human monocyte-macrophage cell cultures and peripheral lymphocytes. In the first case, the level of cytotoxicity was at the border between non-cytotoxic and cytotoxic and in the second test the result was moderate cytotoxic.

1.09.A.T-03 Nanoplastics in Terrestrial Ecosystems: Linking Exposure to Effects Through Dose-Response Assays and Toxicokinetic Modelling

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To date, many studies have focussed on the toxicity of nanoplastics (NPs) on aquatic species, yet there is little information available about their impacts on terrestrial organisms. Moreover, studies which have exposed terrestrial organisms to NPs often could not fully assess particle ingestion due to the methodological difficulties of NPs identification and quantification. Here, we achieve NPs quantification within arthropods with Pd-doped NPs, which are detected via ICP-MS and can be quantified at magnitudes of orders lower concentrations than previously observed in NPs research. We investigated the bioaccumulation, trophic transfer and toxicity of NPs to terrestrial organisms through a suite of assays and toxicokinetic modelling. First, to compile a dose-response curve, we exposed the arthropod detritivore *Folsomia candida* to NPs (0-10,000 mg/kg) through spiked feed for 28 days, assessing toxicity via biological endpoints comprising growth, reproduction, and mortality. Subsequently, feeding studies were performed to create a one-compartment model of NP ingestion and depuration over one month. This enabled the calculation of bioaccumulation factor (BAF) and NP half-life within both *F. candida* and the predatory arthropod *Dalotia coriaria*. Finally, the biomagnification potential of NPs was investigated, by feeding *F. candida* to *D. coriaria*, quantifying NPs within organisms at two trophic levels of a terrestrial food chain and calculating the resulting biomagnification factor (BMF). We successfully quantified NPs ingestion within organisms; NPs LOD was 520 mg/kg (0.05% w/w), with a difference in uptake observed between *F. candida* and *D. coriaria*. While no significant effects were observed on biomarkers in the concentration range tested, NPs half-life and BAF within *F. candida* (9.4 days and 0.033, respectively) suggest short-term bioaccumulation within organisms. Uptake and depuration of NPs has been quantified in *D. coriaria*, and subsequent BAF and BMF will be presented alongside these results. Through this work, we demonstrated the utility of Pd-doped NPs in quantifying the bioaccumulation of NPs at trace concentrations within organisms, and in enabling dose-response ecotoxicology studies for NPs. Furthermore, we elucidated the risks of NPs to terrestrial organisms beyond growth and reproduction. Collectively, these results on exposure and impact will contribute to the assessment of risks of NPs to terrestrial and agricultural systems.

1.09.A.T-04 Emission of Microplastics to Water, Soil and Air – What Can We Do About It?

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Microplastics are plastic particles smaller than 5mm. Sources of microplastics are wear and tear of plastic products (such as car tyres), intentional production (such as rubber infill on sports fields) and fragmentation of macroplastics in the environment. Microplastics in the environment are a concern for human and environmental health. In order to understand the release of microplastics to the environment their material flows from all major sources need to be quantified. A dynamic probabilistic material flow analysis (DPMFA) model [1] was developed for estimating the flow of plastics through the anthroposphere. Micro- and macroplastic emissions to the environment were estimated for the Netherlands and the EU27, for 13 polymers and 7 product categories. The model was used to estimate plastic emissions to soil, air and water compartments with 2019 as reference year. 23 measures to reduce plastic emissions were selected based on insights from literature and experts. The effect of the mitigation measures was estimated for potential for emission reduction in 2030 and 2050, assuming the measures take effect in 2025. The three largest sources of plastic emissions to the environment in the EU27 and the Netherlands are tyre wear, macroplastics and pre-production pellets. The mitigation measures with the largest reduction potential relative to total plastics emission were for the largest sources of plastic emissions, which is to be expected. Furthermore, some of the measures aimed at smaller sources were very effective, but only relative to the plastic emission per source. The results help policy makers prioritize further development of mitigation measures. This model can be extended to other EU countries and the data can be improved. It can support the integrated assessment of micro- and macroplastics mitigation options. [1] Quik JTK, Hids AR, Steenmeijer MA, Mellink Y, van Bruggen A. 2024. Emission of Microplastics to Water, Soil and Air What can we do about it? RIVM report 2024-0106.

1.09.A.T-05 Microplastic-Microbiome Interactions in Aquatic Ecosystems: A Complex Story

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As pervasive and persistent pollutants, microplastics exist alongside diverse microbial communities in nature. These hardy substrates accumulate rich polymeric matrices from their surroundings that in turn offer nutrition and protection to diverse communities and their theatre of activity, representing a new ecological niche. With our work, we aim to establish a holistic understanding of the interactions between microplastics and microorganisms within aquatic systems, and how these interactions shape their surrounding environment. In our research, we combine field sampling with in situ experiments, and apply high-throughput sequencing technologies to 16S genes and whole bacterial genomes. We demonstrate through our studies that biofilm communities exhibit no specificity to plastic polymer type but appear rather to be opportunistic colonizers more strongly shaped by spatial and temporal factors. While the polymer surface does not appear to elicit a selective effect on associated biofilms, our studies show that microplastics might shape communities in a different way. Based on our detection of carotenoid synthesis pathways and photosynthetic gene clusters among plastic biofilms, we postulate this new niche to shape a specific functional toolkit, adaptive among its members as a product of prolonged exposure to UV radiation at the sea's surface. To fully assess the interactions between microplastics and microorganisms, however, one cannot consider plastics singularly but as a component within a more complex and interconnected system. It is known, for instance, that plastics can sorb significant concentrations of polycyclic aromatic hydrocarbons (PAH) in the environment. We show, in our work, that the PAH sorption patterns of microplastics correlate significantly with the structure of associated biofilm communities. Elevated concentrations of specific PAHs on microplastics coincided with the enrichment of selected taxa reportedly capable of hydrocarbon utilisation as well as a reduced diversity among associated biofilm communities. Here, we present a synthesis of our collective research on microplastic biofilms in the aquatic environment, the factors that shape them, and their interactions with nature. To fully comprehend how microplastics impact ecosystems, the entire substrate must be considered, including all chemicals integrated into the polymeric matrix as well as associated biofilms.

1.09.B Impact of Micro- and Nanoplastics on Environmental and Human Health: Monitoring, Fate, Exposure, Toxicity and Mechanisms of Toxic Action

1.09.B.T-01 The Plastisphere Resistome in Antarctic Collected Microplastic Samples: Impact on Human Health

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Microplastic waste has been found in all environments investigated so far, even reaching remote areas such as Antarctica. Microplastics may serve as reservoir and even help spreading to other environmental compartments the bacterial community growing on them (plastisphere). The plastisphere may have antibiotic resistance bacteria within its members that harbour antibiotic resistance genes (ARGs) contributing to the spread of them, which at present, is a human health concern. ARGs are usually carried within genomes by mobile genetic elements (MGEs). There is a scarcity of studies tackling these issues in microplastic waste from remote sites, particularly in the polar areas. The objective of this study is to characterize for the first time the whole resistome (defined as all the ARGs in communities of both pathogenic and non-pathogenic bacteria) found in the plastisphere of plastic samples collected in Antarctica. In addition, the microbial composition of the antarctic plastisphere is also being characterized to perform correlation analyses between ARGs, MGEs and the underlying bacterial diversity that may shed light on the potential role of microplastics in environmental dissemination of antibiotic resistances and their impact on human health.

Sampling took place in King George Island (Antarctic Peninsula) at three sites: Lake Uruguay (plastic, water and soil samples), Lake Ionosférico (plastic, water and soil samples) and the Ardley peninsula

(plastic and soil samples). Resistome analysis was performed by way of SmartChip Real-Time PCR System (TAKARA), which covers 310 ARGs and 72 MGEs. The diversity of the samples was analyzed through metabarcoding of 16S rRNA. 294 unique ARGs and 52 different MGEs were detected across all samples. Globally, plastic samples presented a 30% of exclusive ARGs/MGEs. ARGs can be classified in 5 categories based on the potential risk they pose to human health. Rank I ARGs are the most dangerous to human health, rank IV the least. Plastic samples always presented rank I ARGs. Plastic waste samples also presented significant correlation otherwise not found in soil and water. Microplastics may be acting as a reservoir and vector of ARGs and MGEs in the Antarctic freshwater ecosystem which may have implications for global human health. The authors acknowledge the financial support by MICINN: PID2020-113769RB-C21/22 and Comunidad de Madrid's government for funding through doctorate grant PIPF-2022/ECO25609 the thesis of JMV. MGP would like to thank the International Association of Antarctica Tour Operators (IAATO) and the Council of Managers of National Antarctic Programs (COMNAP) for the joint IAATO/COMNAP Antarctic Fellowship 2020.

1.09.B.T-02 Toxicity of Aged and Fresh Polylactic Acid Nanoplastics on *Pseudomonas putida*
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Introduced in the mid-20th century, plastics have become ubiquitous in modern society. However, the increase in production around 400 million tonnes per year has led to significant environmental impacts. Biodegradable plastics and biopolymers have emerged as potential alternatives, but the environmental impact of biodegradable nanoparticles (NPs) remains largely unexplored. A suitable model organism to study the effects of biodegradable plastics on soil ecosystems is the bacterium *Pseudomonas putida*, a common soil-dwelling species. When exposed to a concentration of 5 mg/L of plastic nanoparticles (NPs) and oligomeric nanoplastics (ONPs), both irradiated (I) and non-irradiated (NI), *P. putida* showed a significant, dose-dependent increase in the production of reactive oxygen species (ROS). In contrast, exposure to ONPs alone induced less pronounced ROS overproduction, especially at the highest concentrations. This difference in ROS overproduction between oligomers alone and the combination of NPs and oligomers may be due to the physical abrasion of the membrane caused by the NPs but not by the oligomeric fraction. Notably, exposure to NPs+ONPs (NI) also resulted in a significant increase in metabolic activity from 5 mg/L, which correlated with increased ROS levels. The association between ROS overproduction and high metabolic activity may partly be explained by *P. putida* attempt to metabolize PLA through the release of non-specific esterases. When exposed to oligomers alone, non-specific esterase activity is generally similar to controls, except for the 1 and 5 mg/L ONPs (I) and 5-10 mg/L ONPs (NI) concentrations, which show a slight increase in metabolic activity. Furthermore, a paradoxical trend was observed: ROS overproduction increased as metabolic activity decreased towards control levels, suggesting an inhibitory effect of irradiated and non-irradiated ONPs at concentrations above 20 mg/L. This inhibitory effect was further evidenced by a decrease in metabolic activity below control levels for both NPs+ONPs (I) and ONPs (I) at the highest concentrations. The aim of this work is to investigate the biological effects of secondary PLA NPs released from PLA microplastics through abiotic degradation on *P. putida* under environmentally representative conditions.

1.09.B.T-03 Multi-Level Approach to Evaluate the Toxicity of Weathered Microplastics From Conventional and Bioplastic Polymers on *Daphnia magna*
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Similarly to conventional plastics, at end-life bioplastic objects can enter and contaminate ecosystems. Once in the environment, plastic objects made of both conventional or bioplastic polymers, can undergo different weathering processes, which can lead to the production of weathered microplastics (wMPs). These wMPs have variable physical-chemical features compared to the virgin ones and might also represent a threat for organisms. Thus, the aim of this study was to investigate the potential toxicity induced by the exposure to wMPs obtained from objects made of conventional (polystyrene - PS and polyethylene - PE) and bioplastics (polylactic acid - PLA and polybutylene adipate terephthalate - PBAT) towards the cladoceran *Daphnia magna*. Weathered MPs were obtained following the ISO 4892-2 regulations, by placing previously obtained MPs inside a solar box. Organisms were exposed for 21-days to three concentrations (0.125 µg/mL, 1.25 µg/mL, 12.5 µg/mL) of weathered PS-MPs, PE-MPs, PLA-MPs or PBAT-MPs. A multi-level approach was applied to investigate adverse effects at different levels

of the biological hierarchy, from sub-individual (molecular and biochemical) to individual and population level. At molecular level, the modulation of the expression of different genes involved in oxidative stress (cat and sod), physiological and behavioral pathways (flot, SERCA and JHE) was assessed. At biochemical level the onset of oxidative stress (activity of antioxidant and detoxification enzymes, and lipid peroxidation) and changes in energy reserves were explored. Changes in body growth and swimming activity were investigated to assess effects at individual level, while reproductive effort was assessed as endpoint at population level. Our results showed that the exposure to WMPs obtained from both conventional and bioplastic objects induced effects at all considered levels of biological hierarchy. Interestingly, the strongest effects were recorded at population level where all the WMPs lead to a modulation in the reproductive effort. In detail, PS and PLA exposure lead to an increase in the number of offspring, PBAT lead to a reduction in the produced newborns and no effect were highlighted for PE. These results suggest the necessity to study in-depth the toxicity of WMPs.

1.09.B.T-04 Developing the Grouping of Polymers to Streamline Toxicology Testing

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Polymers are used across various industries, in everything from medical devices and packaging to automotive components and electronics. However, their diverse manufacturing processes, chemical structures, interactions with other substances, complex degradation processes, and limited toxicity data, make their hazard assessment complicated. Given the impracticality of individual animal testing for each polymer due to cost, time and animal use, there is a critical need to streamline toxicity assessments to better manage the environmental and health risks. Grouping is an approach that streamlines hazard assessment by providing evidence of similarity between substances so that animal hazard data can be read-across (shared) from one group member to another. This poster introduces a novel strategy integrating the existing grouping framework (from the EU project GRACIOUS) by replacing nano-specific elements with polymer-specific considerations. A polymer grouping hypothesis template has been developed which includes consideration of the purpose of grouping (e.g. regulatory), the lifecycle of polymers (to identify relevant exposure scenarios), and answers the fundamental questions of what they are (physicochemical characteristics), where they go (fate and behaviour in the environment, toxicokinetics), and what they do (hazards). The developed hypothesis template used to determine the dermal exposure to Polyacrylic Acid (PAA) in household detergents with a molecular weight of 4500 Da does not imply skin sensitization. The findings support the acceptance of the hypothesis, showing that PAA exhibits low dermal toxicity, no sensitizing potential, and mild eye irritation in rabbits. These results suggest a minimal human health risk through skin contact. It demonstrates the practical application of the framework and highlights future directions for research and implementation.

1.09.C Impact of Micro- and Nanoplastics on Environmental and Human Health: Monitoring, Fate, Exposure, Toxicity and Mechanisms of Toxic Action

1.09.C.T-01 Nanoplastics Selectively Bind and Unveil Novel Ragweed Pollen Allergens

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The pollen of *Ambrosia artemisiifolia*, ragweed pollen, is widespread cause of respiratory allergic reactions. The aim of the work was to provide evidence of corona formation and its properties on NP particles in simulated lung fluids (SLF), simulating event of pollen and NP co-exposure through respiration. *A. artemisiifolia* pollen is chosen as model system for investigation of NPs interactions with respiratory allergens. Its extract was prepared under mild conditions and short exposure to water, simulating rainfall burst of pollen particles. The binding of *Ambrosia* pollen allergens to NPs (PP and PET, 100 nm) was monitored in simulated lung fluids (SLF) by corona formation and mass spectrometry, while the effect of NPs on *Ambrosia* pollen proteins structure was studied by CD spectroscopy. Corona formation was analyzed by glycoproteins staining and immunoblotting with *Ambrosia* pollen allergic patients sera. Relevant NPs binding proteins of ragweed were also identified by shotgun proteomics in subpollen particles. *Ambrosia* pollen NPs corona is composed of specific allergenic proteins in SLF conditions showing highly selective binding of ragweed pollen proteins from the extract. Composition of soft and hard coronae is different between nanoPET and nanoPP particles. Hard corona of nanoPP is dominated by a low Mw glycopeptide(s). We have confirmed that 28-32 kDa proteins of hard corona are PCC13-62-like protein and plant basic secretory protein. We have also confirmed their presence in

subpollen particles. Specific adsorption of novel ragweed proteins and glycopeptides to NPs hard corona have been shown, allowing us to suggest those IgE-binding proteins and peptides to be major biocorona component of NPs. Our data provide insight into molecular events occurring during co-exposure of NPs and allergens. Selective binding of allergenic proteins to NPs may during co-exposure change the route of allergen exposure to the immune system and modulate allergic reactions. Funding from Horizon 2020, No965173, IMPTOX, by Ministry of Science, Innovation and Technological Development, Republic of Serbia, grant No451-03-66/2024-03/200168 and Serbian Academy of Sciences and Arts, GA F-26.

1.09.C.T-02 Polyvinyl Chloride- and Polypropylene-Model-Micro- and Nanoplastics Exhibit Different Mechanisms of Toxicity in Human Umbilical Vein Endothelial (HUVECs) Cells

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Plastic pollution, particularly in the form of micro- and nanoplastics (MNPs), has become a significant concern for both environmental and human health. Alarmingly, MNPs have been detected in human placental tissue, raising serious concerns about potential maternal-fetal exposure and its implications for fetal development. It is expectable that these small particles in the maternal bloodstream can be transferred to the fetal circulation and influence children's future lives. Since Polyvinyl chloride (PVC) and polypropylene (PP) are dominant plastic types in environmental samples, we used model-MNPs made of these polymers labeled with fluorescent-quantum dots (qDs). To investigate the effects of these potentially circulating polymers at a fetal level, we used Human Umbilical Vein Endothelial Cells (HUVECs) as cell model, as representative of structural and biological barrier. Cytotoxicity assays revealed a concentration- and time-dependent decline in cell viability, with PP exerting a more pronounced effect, particularly at higher concentrations and extended exposure. Flow cytometry and confocal microscopy analyses further uncovered differential cellular responses. PVC-treated cells exhibited significant morphological changes, characterized by increased complexity and granularity, alongside elevated colocalization of MNPs with cellular membranes. In contrast, PP-treated cells demonstrated lesser internalization and predominantly surface-associated interactions. Z-stack fluorescence analysis confirmed the intracellular accumulation of PVC MNPs, with their peak fluorescence intensity outpacing that of PP across cellular layers. These findings suggest that the physicochemical properties of PVC and PP, such as surface charge and hydrophobicity, drive their differential interaction patterns. While both MNP types penetrate cells, PVC exhibits stronger affinity and uptake, potentially posing greater risks to endothelial integrity and vascular health. This study provides critical insights into the cellular effects of common environmental MNPs, emphasizing the need for further investigation into the biological effects and mechanisms of nanoparticle interactions with vascular cells to better assess their risk in maternal and fetal contexts. Ministry of Health, Italy by IRCCS - Burlo Garofolo (RC38/23)

1.09.C.T-03 Distribution of Micro- and Nanoplastics in Perfused Human Placental Tissue

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Although micro- and nanoplastics (MNPs) have been found in numerous environmental and human samples, their fate and toxicity is still mostly unknown. As in utero exposure to environmental pollutants may adversely affect fetal health, assessing risks of MNPs to the unborn child is of utmost importance. Ex vivo placental perfusion is a unique system to gain insight into MNP particle size- and type-specific uptake, transport, localization, and quantification. Placentas were attached to a perfusion chamber with both fetal and maternal circulation. The placenta was perfused for a maximum of 6 h with 25 µg/mL of 50 nm, 200 nm, or 1 µm fluorescent polystyrene commercial spheres. Tissue pieces from before and after perfusion were collected, as well as the liquids in the maternal and fetal reservoir before and after perfusion. The perfused tissue was cryo-cut in slices of 30 µm thickness, and the slices were stained using SiR-actin to stain the actin components of the cells. The stained tissue slices were then analyzed with a Confocal Fluorescence Microscope (CFM). The perfusion solutions were measured with a spectrophotometer. Fluorescent polystyrene particles of all three sizes were found to be present in perfused placental tissue. The 50 nm and 200 nm particles seemed to agglomerate around the villi in the placental tissue, where transfer between maternal and fetal sides takes place. The 1 µm particles did not cluster and were found inside the villi. Fluorescence intensity measurements of the perfusion solutions showed limited transfer of all particle sizes to the fetal circulation, but loss of particles on the maternal

side, suggesting significant retention in tissue. MNP transport between maternal and fetal side and MNP uptake by placental tissue were studied. All three particle sizes used were found back in the perfused tissue. Smaller particles exhibited a greater tendency to agglomerate around villi compared to larger particles. The perfusion solution on the fetal side showed increased fluorescence after perfusion with 1 μm particles, indicating that a part had crossed to the fetal side, as is also observed with CFM. Smaller particles were retained in the tissue to a greater extent, i.e., 92% and 63% were retained for 50 nm and 200 nm, while only 26% of the 1 μm particles were retained. Next steps include the prevention of clustering of smaller particles during perfusion, as well as perfusions with other MNP polymer types synthesized by our research group. This project has received funding from the European Union's Horizon 2020 research and innovation programme under AURORA grant agreement No 964827.

1.09.C.T-04 Probabilistic Human Risk Assessment of Micro/Nanoplastics: Integrating the Aggregate Exposure Pathway and Adverse Outcome Pathway (AEP-AOP)

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Microplastic particles are ubiquitous in the environment, found in everything from the air we breathe to the food we eat. Despite progress in microplastic research, evaluating the human health risks of micro- and nanoplastics (MNPs) remains challenging due to their varied characteristics and multiple pathways. This study aims to assess the human health risks of MNPs by applying an Aggregate Exposure Pathway and Adverse Outcome Pathway (AEP-AOP) framework integrated with a Bayesian network, taking into account the material properties and multi-media characteristics of MNPs. To enhance the risk assessment of MNPs on human health, several key methodologies were adopted. First, a quality assurance and control (QA/QC) screening process was implemented to ensure data reliability. QA/QC criteria filtered non-standard exposure and toxicity data from the literature, and only datasets meeting these standards were included in the analysis. Next, data alignment methods using parameterized probability density functions (PDFs) were applied to better reflect real-world conditions. Exposure data were standardized by size range, while toxicity data were adjusted to reflect the polydispersity of environmental MNPs and biologically relevant size fractions. Following data alignment, a Cumulative Risk Assessment (CRA) was conducted. An AEP-AOP network was constructed from a comprehensive literature review and the AOP wiki database to integrate the diverse exposure pathways of microplastics. Key event relationships were further assessed using a Bayesian network modeling approach, which improved the network's reliability by analyzing probabilistic relationships. Based on the AEP, total daily exposure to MNPs was calculated, and Margin of Exposure (MoE) values were derived for each key event within the AOP to assess potential risks. This study provides a robust framework for next-generation human risk assessments of MNPs, utilizing an AEP-AOP approach integrated with Bayesian networks to address the complex properties of MNPs through various advanced methodologies. Additionally, the NGRA results identified oral ingestion as the primary human exposure route, underscoring potential developmental risks associated with MNP exposure. These findings emphasize the need for further in-depth research to examine the multi-media exposure pathways and developmental toxicity of MNPs. This work was supported by Korea Environment Industry & Technology Institute (KEITI) through Technology Development Project for Safety Management of Household Chemical Products, funded by Korea Ministry of Environment (MOE)(RS-2023-00215309)

1.09.C.T-05 Biological Evaluation of Binding of Toxins to Microplastics

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Micro- and nanoplastics (MNPs) contaminate ecosystems worldwide and enter our food and water via the aquatic environment or plastic food containers. MNPs could serve as carriers of aquatic or food pathogens and/or their toxins, facilitated by their large surface area and hydrophobic nature. However, there is currently no method for qualitative or quantitative evaluation of toxin binding to MPs. This study focuses on cereulide (CER) and fumonisin B1 (FB1) as model toxins, examining their binding to microplastics (MPs) composed of polyethylene terephthalate (PET) and polypropylene (PP) under simulated environmental and biological conditions. It aims to evaluate the binding of toxins to MPs by assessing the subsequent biological effects using advanced cell-based models and analysis techniques. The work is part of the EU-funded IMPTOX project, aiming to unravel the mechanisms of MP-toxin interactions and their implications for human exposure and toxicity. An optimized 3D in vitro cell model assisted in exposing hepatic HepG2 spheroids to the different conditions. The spheroids were chronically exposed to MPs that

were pre-incubated for 2 hours with the toxins. Using IncuCyte Sx5 Live Cell Analysis and Agilent Seahorse extracellular flux analysis, the cells' bioenergetic responses were monitored. Shifts in metabolic parameters for bound toxin-MPs versus the MPs or toxins alone indicate that this biological approach could serve as an effective proxy to investigate toxin-MP binding. This research has been funded by IMPTOX European Union's Horizon 2020 research and innovation program (grant number 965173), Research Foundation Flanders research grant 1506419N, Ghent University Special Research Fund grant BOF20/BAS/120, The Federal Public Service (FPS) Health, Food Chain Safety and Environment project RT 21/5 CYANTIR and Research Foundation Flanders/ Slovenian Research and Innovation Agency Weave project with grant number G000123N. M.F.A. received a postdoctoral mandate funded by Ghent University Special Research Fund (BOF) grant number BOF01P03220.

1.09.P Impact of Micro- and Nanoplastics on Environmental and Human Health: Monitoring, Fate, Exposure, Toxicity and Mechanisms of Toxic Action

1.09.P-Mo058 Micro- and Nanoplastics Libraries Reflecting Environmental Physicochemical Properties

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Microplastics (MPs), defined as plastic particles smaller than 5 mm, are found widely across the environment, while nanoplastics (NPs) are classified as particles under 1 µm in size. The recent discovery of MPs in human tissues such as the lung, placenta, and blood underscores their human exposure through inhalation or ingestion. MPs and NPs (MNPs) are also prevalent in diverse environments, including air, rivers, and even drinking water. However, their full impact on human health remains largely uncertain. Although MNPs exhibit varied physicochemical properties (e.g., size, shape, and surface oxidation induced by environmental factors like UV radiation), research often overlooks these complexities when evaluating their biological effects. Consequently, particles representing environmentally relevant MPs and NPs are scarce, with most studies relying on plastic particles with limited physicochemical diversity due to availability. To address this gap, we are working on development of MNPs libraries that consider size, shape, surface oxidation of environmental MNPs. We selected polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyethylene terephthalate (PET) as target polymers based on their high global production. Fragmented particles for each polymer, ranging from NPs to MPs in size, were prepared. In addition, surface-oxidized MPs (oxiMPs) were produced by irradiating them with 172 nm vacuum UV light in air, simulating environmental conditions. For comparison, environmental MPs were collected from a sandy beach along Osaka Bay. Analytical techniques such as ATR-IR revealed hydroxy and carbonyl groups on both the environmental MPs and oxiMPs. To create smaller particles (NPs, under 1 µm in diameter), we employed a previously reported precipitation-based method. Scanning electron microscopy confirmed the successful production of NPs particles. In order to kinetics analysis, we used Nile Red to tag each MNPs, achieving successful labelling, and further adapted the protocol to incorporate Qdot, a bright and photostable fluorescent label, for NP labelling, validated via microscopy. These MNPs libraries, which reflect complex physicochemical properties, enhance the applicability of MNPs research to real-world environmental contexts. They are available for collaborative distribution upon request. This research was performed by the Environment Research and Technology Development Fund (JPMEERF20241003) of the Environmental Restoration and Conservation Agency provided by Ministry of the Environment of Japan and the Sumitomo Foundation for Environmental Research Projects.

1.09.P-Mo059 Detection of Internalized Nanoplastics in Human Cells Using Surface-Enhanced Raman Scattering (SERS)

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Nanoplastics (< 100 nm) represent one of today's most pressing environmental and health challenges. Compared to larger plastic particles, nanoplastics exhibit increased bioavailability, which can significantly elevate ecological and human health risks. Despite their widespread occurrence, the understanding of how

these particles interact with biological systems remains limited. The detection, monitoring, and characterization of nanoplastics in biological contexts, especially in human cells, is a significant challenge due to their size and low environmental concentrations. This study aims to address this gap of imaging and detecting nanoplastics at relevant concentrations within human cells using Surface-Enhanced Raman Spectroscopy (SERS). SERS is an analytical technique capable of providing molecular-level information with high sensitivity and spatial resolution, making it suitable for detecting and visualizing nanoplastics at low concentrations in complex biological environments. To investigate the viability of SERS as a tool for detecting nanoplastics internalized in cells at low concentrations ($<100 \mu\text{g/L}$), we used macrophages (THP-1 PMA-differentiated) as a cell model. The cells were sequentially exposed to spherical AuNP, followed by exposure to polystyrene (PS) nanoplastics (100 nm) as test nanoplastics. SERS measurements were conducted with a 633 nm laser wavelength in a WiTec Alpha 300 R confocal Raman microscope. Raman mappings collected with enhanced vibrational signals from SERS enabled the identification of AuNP and PS nanoplastics within the cellular environment, confirming the uptake of both particles by the cells. Our findings demonstrate the effectiveness of the SERS technique for detecting and intracellular localization of nanoplastics in human cells and potentially across various cell types. This dual capability for detection and imaging underscores the utility of SERS in studying cellular interactions with nanoplastics. These results contribute to a foundational understanding of nanoplastic behavior, interactions within cells, and their potential health impacts. The authors thank the São Paulo State Research Support Foundation (FAPESP) (Process 2024/07778-1) for financial support to the project.

1.09.P-Mo060 Linear Polyethylene Terephthalate Oligomers Toxicity in Human Primary Cells and Interactions with Biomolecules

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Side-products of PET polymerization reactions are cyclic and linear oligomers of PET, recently recognized as novel non-intentionally added substances in food (NIAS) and classified as poor data chemicals. Risk assessment of PET oligomers is challenging due to their size, number, isomerism, complexity in structure, and lack of analytical standards. The great heterogeneity in size and structure of PET oligomers calls for a systematic approach in risk assessment and hazard identification. The aim of our study was to synthesize and characterize physicochemical properties, to investigate solubility in common solvents and food simulants, as well as protein, DNA and cellular interactions of a series of linear methylated and non-methylated PET oligomers (monomer, dimer and trimer). Our results show striking differences in the properties of PET oligomers in relation to size and end-group chemistry (methylated vs. free carboxyl- vs. hydroxyl-). Solubility in food simulants decreases with increase in methylation and the number of aromatic rings. Linear PET oligomers show little to no toxicity in a wide range of concentrations tested in primary cells. All linear PET oligomers tested readily interact with food and serum proteins, and with salmon sperm DNA. Our results point to the importance of size and end-group of PET oligomers in chemical risk assessment: size and methylation of the oligomer strongly contribute to the observed cellular and molecular effects of tested compounds. PET oligomers binding to DNA prompts further research on the toxicological relevance of the observed interactions. This study was supported by the Serbian Academy of Sciences and Arts Project F-26; IMPTOX European Union's Horizon 2020 research and innovation program (grant number 965173); the Ministry of Science, Innovation and technological development of the Republic of Serbia (Contract number: 451-03-68/2024-14/200168). This research was supported by the Science fund of the Republic of Serbia, Grant No. 7275, Exploration of PETase side activity of digestive enzymes of human gastrointestinal tract acting on micro- and nanoplastics: mode of action and products characterization XPACT and Region Stockholm (ALF project FoUI-986234), The Swedish Cancer and Allergy Foundation, The Swedish Asthma and Allergy Association's Research Foundation (Number: F2022-0011); The Swedish Heart-Lung Foundation (Number: 2021042), The Konsul Th C Bergh Foundation, The Magnus Bergvall Foundation and The Karolinska Institutet's Research Foundation Grant.

1.09.P-Mo061 The Toxicity of Microplastics Explorer (ToMEx) 2.0 Database – A Unique Compilation of Microplastics Effect Measurements for Environmental Risk Assessment

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The Toxicity of Microplastics Explorer (ToMEx) database is an open-source, open-access database and web application for microplastics toxicity. Its purpose is to provide a valuable easy-to-access tool for data exploration, visualization, and analysis, and a data source for the development of environmentally safe thresholds for micro- and nanoplastic exposure. However, since its release three years ago, the peer-reviewed literature has continued to expand exponentially, rendering ToMEx increasingly obsolete. To guarantee the continued utility of ToMEx, a crowdsourcing approach was employed to establish a global workgroup tasked with updating ToMEx by extracting data from additional studies published after the original release. We here present the results and the experience with this process, which led to ToMEx 2.0 now having doubled in size. Although the aquatic organisms database now encompasses a greater diversity of species and test particle characteristics, both the aquatic and the human health database remain biased towards a few dominant particle characteristics (polystyrene spheres) and particle-only studies (without additional chemicals, not leachates). To investigate, whether the increased amount of data can be used to derive more precise regulatory thresholds, a previously developed framework for establishing health-based microplastic thresholds for the state of California was reapplied. We show that thresholds for ToMEx 2.0 are lower and more precise than earlier thresholds based on ToMEx 1.0. Nevertheless, the data suitable for threshold development remained limited with only few proper dose-response experimental designs added to derive No Observed Effect Concentrations (NOECs) and the shift was driven mainly by two new publications. Given the effort required to update the ToMEx database, we believe that future updates should only incorporate studies that meet strict quality criteria to ensure data utility and ensure feasibility. Nevertheless, ToMEx remains a valuable tool for the research community, and this exercise demonstrates that large, coordinated data-mining efforts are feasible. Funding for this project was provided by the Southern California Coastal Water Research Project Authority. B.C.A. is grateful for funding from the Swedish Research Council for Sustainable Development FORMAS 2018 01201. M.M.M. received funding from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) SFB 1357 391977956.

1.09.P-Mo062 Exploring the Toxic Effects of Microplastics and Heavy Metals on *Enchytraeus crypticus*: A Threat to Soil Ecosystem Integrity

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Polypropylene (PP), polyethylene (PE), and polystyrene (PS) are among the most produced plastics globally. Once released into the environment, these plastics degrade into microplastics (MPs) that infiltrate water systems, soil, and ecosystems, potentially exerting toxic effects on organisms. Beyond their inherent toxicity, MPs can modify the effects of co-occurring pollutants such as heavy metals by acting as vectors, providing surfaces for metal adsorption. This interaction can significantly influence the bioavailability and toxicity of metals, including cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni), copper (Cu), and zinc (Zn), to soil organisms. These metals, when present in soil, pose serious risks to ecosystem and human health as they may accumulate in plants and enter the food chain. This study assessed the combined effects of PE, PS, and PP microplastics with Cd, Cr, Pb, Ni, Cu, and Zn on the reproduction of the soil organism *Enchytraeus crypticus*. Reproductive toxicity was quantified through EC50 values to evaluate individual and combined toxicities. Results revealed that when combined with individual microplastics, all metals exhibited synergistic effects. The EC50 for Cd and MPs was found to be 8.2 mg/kg, representing the most toxic interaction, while Zn and MPs had an EC50 of 97 mg/kg, showing the lowest toxicity among the metals. Notably, when all six metals and all three microplastics were present together, the EC50 was significantly reduced to 2.34 mg/kg, indicating a remarkably strong synergistic effect. These findings highlight the critical role of MPs in enhancing the toxicity of heavy metals through vector effects, particularly in complex mixtures. The observed interactions underscore the importance of considering mixture toxicity in environmental risk assessments. Understanding the combined impacts of priority pollutants such as MPs and heavy metals is essential for evaluating their ecological risks and guiding the development of effective environmental regulations to protect soil ecosystems. This study was supported by the SETAC Europe 2025 Registration Grant - SETAC Europe

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1.09.P-Mo063 Breaking Down Recycled LDPE: Nanoplastics, Byproducts and Their Effects on aquatic organisms

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The increased use of recycled plastics offers a promising approach to mitigate the environmental accumulation of plastic waste. However, it remains unclear whether these plastics, once released into the environment, generate nano-range particles and whether such nanoplastics or the additives they may release pose a potential threat to environmental and human health. This study aims to evaluate the toxicological effects of secondary nanoplastics (NPLs) derived from recycled low-density polyethylene (LDPE) plastics and their byproducts on key aquatic organisms. Three representative species were selected for testing: the green microalga *Chlamydomonas reinhardtii*, the freshwater plant *Spirodela polyrrhiza*, and the water flea *Daphnia magna*. Secondary nanoplastics were produced by mechanically fragmenting LDPE microplastics, and their presence was confirmed through extensive physicochemical characterization using dynamic and electrophoretic light scattering, scanning electron microscopy, and Fourier transform infrared spectroscopy. Each organism was exposed to increasing concentrations of secondary nanoplastics and released by-products to assess their biological effects. Acute exposure to both secondary LDPE-NPLs and LDPE-Byproducts had a significant effect on *Daphnia magna*, with 40% of individuals being immobilized after 48 hours of exposure at 100 mg/l. The ribosomal protein Rpl32 and the one encoding the antioxidant enzyme CuZnSOD have been significantly repressed in the presence of LDPE-NPLs and LDPE-Byproducts. Significant changes have been observed in the expression of glutathione S-transferase, insulin receptor and ecdysone-responsive genes, potentially affecting the molting and other intracellular processes. Acute exposure (96h) to secondary LDPE-NPLs alone had no significant effect on *Spirodela polyrrhiza* in the range of concentration between 1 to 100 mg/l. However, effects were observed with concentration as low as 1 mg/L in various physiological parameters when the duckweed was exposed to LDPE-Byproducts. By examining the effects on these aquatic species, this research highlights the potential environmental risks associated with recycled plastics. It provides critical data on the behaviour and toxicity of secondary nanoplastics, contributing to a deeper understanding of the ecological implications of plastic recycling. This research was supported by 2020-113769RB-C21/22 and RYC2021-034953-I grants from MICINN.

1.09.P-Mo064 Effect of Polyethylene Microbeads on the Growth and Photosynthetic Potential of Freshwater Microalgae, *Scenedesmus quadricauda*

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Microalgae are primary producers in food chain and disruption of microalgal population can have cascading effect to every trophic level in the ecosystem. Currently, the background concentration of microplastics in the freshwater systems is increasing which could have potential harmful effect on microalgae. A systematic study is required to investigate the potential toxicological effects of microplastics on microalgae. In this study, the effect of frequently observed microplastics, specifically polyethylene, on a common freshwater microalga, *Scenedesmus quadricauda* was investigated following the OECD 201 guidelines for the freshwater alga and cyanobacteria growth inhibition test. An incubator shaker equipped with custom-built, bottom-illuminated photobioreactor array is used for the study. Exponentially growing culture of microalgae are exposed to different sizes and concentrations of the commercially available polyethylene microbeads. After every 24 hours, the cell counts, growth rate and photosynthetic efficiency are determined and compared to unexposed control samples. Furthermore, at the end of 72 hours, the microalgae are harvested to study the potential effects on photosynthetic pigment concentration. The microalgae may form biofilm over/with microbeads and size dependent effect could be observed. Larger sized microbeads could provide a surface for microalgae to form biofilm whereas smaller sized microbeads could cover the microalgal surface triggering shade adaptation. Additionally, effect of environmentally relevant and higher concentrations in an isolated system will help determine the effective concentration for growth inhibition for each particle size tested. Lastly, this study aims to utilise the results to draw implications for environmental monitoring studies on microplastics and to provide evidence for future ecotoxicologists and policy makers regarding effect of microplastics on freshwater microalgae. This work is funded by Research council of Finland (Academic project, Decision No. 347690, Awarded to Amit Bhatnagar)

1.09.P-Mo065 Interaction of Aged Filamentous and Fragmented Polypropylene Microplastics in Two Freshwater Zooplankton Species

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Microplastic (MPs) pollution represents a ubiquitous threat to aquatic ecosystems, with numerous studies highlighting the detrimental effects of pristine MPs on aquatic organisms. Unlike pristine MPs, aged MPs exhibit altered surface properties, chemical composition, and interaction dynamics with organisms, making their study essential to understanding the environmental impacts. Despite their high ecological relevance, experiments using environmentally aged MPs remain limited. This research addresses this critical gap by investigating the interactions between aged filamentous and fragmented polypropylene (PP) MPs and zooplankton, focusing on entanglement, ingestion, and physiological responses, to provide a more comprehensive understanding of the ecological impacts of aged MPs. Aged PP MPs were obtained from 20-year-old fishing gear recovered from the Adriatic Sea, mechanically broken down and processed into filamentous and fragmented forms and characterized. Neonates of two zooplankton species, *Daphnia magna* and *Thamnocephalus platyurus*, less than 24 hours old, were selected for their distinct differences in size, morphology, feeding strategies, and ecological roles. The neonates were exposed to increasing concentrations of filamentous (0–50 items per well) and fragmented MPs (0–1000 items/mL) over 24 hours. Endpoints included survival, locomotory dynamics, heart rate, feeding behaviour, ingestion rates, and total copper content, assessed via microscopy, flow cytometry, and ICP-MS. The results demonstrated that filamentous MPs significantly reduced locomotion and promoted entanglement in *D. magna* across higher concentrations tested (5–50 items/mL), with altered swimming speed and movement trajectories observed in entangled individuals. In contrast, algal ingestion by *D. magna* was not affected by filamentous MPs. Fragmented MPs exhibited concentration-dependent entanglement in *D. magna*, with ingestion quantified by copper content. Entanglement with *T. platyurus* was absent in both MPs shapes. The findings highlight the role of organismal morphology, body size, and MPs characteristics in mediating interactions. These findings underscore the importance of incorporating aged environmental MPs in toxicity studies, to provide ecologically relevant insights. These findings enhance our understanding of the morphological and behavioural impacts of MPs on zooplankton, advancing knowledge of their long-term ecological consequences in aquatic environments.

1.09.P-Mo066 Investigating the Sensitivity of *Daphnia magna* to Natural and Synthetic Microfibers

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Microfibers from textiles are one of the most abundant anthropogenic microplastics (MPs) found in environmental samples, with research suggesting most microplastics ingested in situ are microfibers. Microfibers released from clothing can be either from synthetic or natural fibers, which may have different ecotoxicological profiles. Research has suggested that microfibers may be more toxic than fragments or beads due to their capacity to penetrate gut walls and comparatively slow excretion rates. However, there is limited information about hazards from natural and synthetic microfibers with even less information about uptake and depuration. The present study investigated acute toxicity, uptake rates, and depuration rates, of synthetic/semi-synthetic (acrylonitrile, polyester, Kevlar, and nylon) and natural (cotton and silk) microfibers towards *Daphnia magna*, a water flea. Microfibers were well characterized with microfibers maintained in suspension counted to assess if biological uptake was linked to fiber stability in test media. Species were exposed to the microfibers in a dose-response (10 to 100,000 particles/L) format to identify lethal thresholds and determine if uptake rates were linked to exposure concentrations. Results demonstrated little to no acute toxicity with all microfibers except for nylon which had a lethal concentration where 50% *D. magna* were expected to die at 0.34 ± 1.26 mg/L. Microfibers used in the study are transparent, making it difficult to identify retention in the body, however results demonstrated that both Kevlar and nylon accumulated at higher rates than other polymer types. Data also suggested that suspension stability was not related to ingestion. This result was supported by the observation of organisms ingested MPs on the bottom of the exposure chamber. Acute toxicity may not be a sensitive endpoint for characterizing the hazards of microfibers. Microfibers which were demonstrated to accumulate in organisms (Kevlar, polyester, and nylon) were investigated for chronic toxicity to characterize alterations to growth rates and reproductive capacity. Results suggested that polymer type influenced ecotoxicity of microfibers. Follow-up research is needed to more appropriately characterize the potential effects of additives and degradation on ecotoxicological potential.

1.09.P-Mo067 Biological Effects of Microcapsules 2

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Microcapsules are the minute particulate matter which encloses various chemical substances inside of the core. The core base for particles are often made by a kind of plastics so it can be regarded as microplastic. They are used in many aspects including drug delivery, stabilizing agricultural chemical effect and to keep fragrancedurability of the aromatic product. The environmental behavior of the chemicals in the capsule changes depending on the properties of the base. It means encapsulation allows any chemical substance to have long-distance mobility and resistance to decomposition results in being equivalent properties of so-called POPs. This may bring the hazard for the environment. On the other hand, this characteristic change of chemicals can be utilized for ecotoxicity testing especially to waterborne animals.

The characteristic of microcapsules provides the change to the behavior of encapsulated chemicals, such effect like easily decomposable chemicals to stable or volatile component to less diffusible. This makes those chemicals difficult to tested can be treated. Also, the exposure process for water animals will be changed from percutaneous to oral intake.

Therefore, with the aim to clarifying the exposure effects of chemicals to organisms mediated by microcapsules, we synthesized microcapsules encapsulating specific chemicals and investigated the changes of the toxicity before and after capsulation. We have synthesized microcapsules with several chemicals and conducted ecotoxicity tests to *Daphnia* and Medaka. The result will be presented.

1.09.P-Mo068 Evaluating the Role of *Sinotaia quadrata* (Benson, 1842) as a Bioindicator of Microplastic Pollution in Tuscany's (Italy) Freshwater Ecosystems

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The Asiatic gastropod *Sinotaia quadrata* (Benson, 1842), an invasive species in European freshwater ecosystems, may serve as a valuable bioindicator of microplastic (MP) pollution in streams affected by human activity. To assess its potential as a bioindicator of MP contamination, sampling was conducted in Tuscany (Italy) in August 2023. Five sampling sites were selected along three rivers (Arno, Bisenzio, and Ombrone) in areas near urban settlements and subjected to significant anthropogenic pressures (such as the presence of industrial textile factories). Samples were collected from both biotic (*S. quadrata*) and abiotic (water, sediment) compartments, with *S. quadrata* specimens categorized into four size classes. Fourier-transform infrared spectroscopy was used to identify and quantify MPs. In total, 84 MP particles were detected, including 56 within *S. quadrata*, 11 in water, and 17 in sediment samples. The predominant colors of MPs were white (28.74%), blue (34.48%), and black (36.78%), and they were composed of fragments (50%) and filaments (50%), with polymers such as polyethylene (PE) (34.48%), polyethylene terephthalate (PET) (29.89%), and polypropylene (PP) (35.63%) being most common. Among abiotic samples, MPs were more abundant in sediment than in water. No significant differences were observed in MP particle size among different *S. quadrata* classes. Smaller individuals ingested a higher number of MPs compared to larger individuals, with a significant negative correlation between mollusc size and MP count. Additionally, the generalized linear mixed model revealed that the number of MP items per gram of *S. quadrata* was predicted solely by mollusc weight. These results could be linked to differences in feeding habits between smaller and larger specimens. These findings indicate that *S. quadrata* accumulates MPs in its digestive tract, especially during juvenile stages, with ingested MPs reflecting the types present in sediment. Given the potential risks of MP ingestion through local human consumption of *S. quadrata*, this species offers a useful tool for assessing MP pollution in sediment-rich freshwater ecosystems.

Additionally, this study highlights the broader application of invasive species in monitoring and managing anthropogenic impacts on aquatic habitats.

1.09.P-Mo069 Ecotoxicological Impacts of Microplastics on the Freshwater Species *Danio rerio* and *Daphnia magna*

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Human activities have introduced a wide range of contaminants into freshwater and marine ecosystems,

including microplastics (MPs), which are persistent and widespread pollutants. MPs pose substantial threats to natural ecosystems, impacting organisms across multiple biological levels. Due to their durability and slow degradation, these particles present ongoing risks to aquatic life. In this study, we accessed the potential effects and environmental hazard of relevant MPs, namely polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), and high-density polyethylene (HDPE), on two aquatic model species, the crustacean *Daphnia magna*, and the fish *Danio rerio*. All four MPs were exposed to both *D. magna* and *D. rerio* at the same concentration range (1 to 100 mg/L). Experiments involving *D. magna*, followed the OECD Test Guideline (TG) 202 for acute immobilization test and, after 48 hours of exposure, immobilization was documented. For *D. rerio* experiments, the OECD TG 236 was followed, and after 96 hours of exposure period, malformations, hatching delay, and mortality were documented. Regarding *D. magna*, no immobilization was observed at the end of the 48 hours of exposure for all tested concentrations of each MP. For *D. rerio*, no mortality, malformations, or hatching delay were observed in embryos/larvae exposed to the different concentrations of the four MPs. These results indicate that, for all measured endpoints in both organisms, the no observed effect concentration (NOEC) is equal to 100 mg/L, demonstrating that the four MPs are no/low toxic for both tested species. The present study provides a better understanding of the interactions between different types of microplastics and various trophic levels as well as biological communities, much needed for a more comprehensive risk assessment. To go further, additional tests are currently being conducted on other model species representative of different aquatic compartments, trophic levels, and life traits to understand if no adverse effects are also recorded on selected species. Thanks are due to the Portuguese Foundation for Science and Technology (FCT) for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020+LA/P/0094/2020) and CICECO (LA/P/0006/2020, UIDP/50011/2020, UIDB/50011/2020), to Rita Fernandes (PRT/BD/154317/2023), through a Doctoral grant, to R. Martins (2021.00386.CEECIND; DOI: 10.54499/2021.00386.CEECIND/CP1659/CT0011) and to the NANOGREEN (CIRCNA/BRB/0291/2019; DOI: 10.54499/CIRCNA/BRB/0291/2019) and DigiAqua (10.54499/PTDC/EEI-EEE/0415/2021) projects. We also acknowledge the financial support of the European Union's Horizon programme through the project SAFERCOAT, under the grant agreement No. 101182588.

1.09.P-Mo070 Interactive Effects of Polystyrene Nanoplastics and Climate Change Stressors on Zebrafish Embryo Development and Behavior

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Nanoplastic prevalence in all environments is a reality that will only worsen with time as more human plastic waste gets released and degraded. Research on the effects of nanoplastics on aquatic organisms has increased in the past years. However, few studies have taken into consideration the potential interaction between these particles and the effects of climate change stress factors despite its importance regarding environmental risk assessment. The present study aimed at investigating how exposure to polystyrene nanoplastics (PSNP) could potentially interact with two important climate change stressors (pH decrease and temperature increase) to disrupt zebrafish embryo development and behavior. We assessed a variety of endpoints: spontaneous tail coiling activity at 24 hours post fertilization (hpf) as a measure of early neuromuscular activity; morphological characteristics at 72 hpf; and behavioral characteristics at 120 hpf (overall activity, anxiety, and habitative learning). Our findings revealed that there are complex interactions between climate change variables and nanoplastic exposure that affect developmental endpoints differently. PSNP exacerbated the effects of temperature increase and pH decrease for some aspects while for others their effect was independent or negligible, underlying the importance of studying multiple endpoints to fully understand the interactive effects. Our results highlight the importance of addressing the interaction between different environmental stressors to predict cumulative and synergic effects on aquatic ecosystems in a rapidly changing environment. Work funded by the Spanish government Grant PID2023-148109OB-I00.

1.09.P-Mo071 Toxic Effects of PET microfibers on Sheepshead Minnow, *Cyprinodon variegatus* Jin Soo Choi¹, Wan-Seob Cho² and June-Woo Park¹, (1)Korea institute of toxicology (KIT), Korea, Republic of, (2)Dong-A University, Korea, Republic of

PET fibers in the environment can be degraded into microfibers because of weathering processes such as sunlight, physical wear, and heat. Although recent studies reported adverse effects of PET microfibers on aquatic organisms, the lack of information on their toxicity and mode of action hampers the risk

assessment of PET microfibers. Therefore, this study aimed to investigate the biological effects of PET microfibers and their underlying mechanisms in early-staged sheepshead minnows (*Cyprinodon variegatus*). PET microfibers (about 13 μ m diameter $\times$ 106 μ m length) were prepared by cutting PET threads and treated to sheepshead minnow larvae at 10 and 100 mg/L for 10 days. No acute toxicity was found in the minnow, but PET microfibers significantly produced reactive oxygen species and reduced behavioral responses of traveled distance and maximum velocity. The transcriptomic data suggested that Merkel cells (flow sensors) and corpuscles of Stannius (calcium regulator) are putative targets, which were derived from oxidative stress, sensory neuropathy, cognitive impairment, and movement disorders. These findings underscore that although PET microfibers are not directly lethal to sheepshead minnows, they could impact their survival by damaging swimming-related key genes. This study provides new insights into how PET microfibers are toxic to aquatic organisms and disrupt ecosystems beyond survival and pathological changes. This research was supported by Risk assessment to prepare standards for protecting marine ecosystem of Korea institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (KIMST-20220383), and by a research project titled "Development of a convergence solution for environmental contamination issues of secondary microplastics" of the National Research Council of Science & Technology (NST) grant by the Ministry of Science and ICT (MSIT), Republic of Korea (No. CAP20024-000).

1.09.P-Mo072 Enhanced Fish Ingestion of Microplastics Due to Colonization and Stimulated Bioluminescence of Luminescent Bacteria on Microplastics

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Microplastics (MPs) are emerging pollutants in marine environments that can interact with various marine organisms, potentially altering their behavior and ecological roles. This study evaluated the effects of polyvinyl chloride (PVC) MPs (~1 mm) on the colonization and bioluminescence of the marine bacterium *Photobacterium phosphoreum* and investigated the predation behavior of the nocturnal fish *Sphaeramia nematoptera* towards glowing and non-glowing MPs under dark conditions. Biofilm formation was significantly higher on UV-aged MPs compared to pristine, ethanol-washed MPs, and natural sand particles, suggesting a preference for aged MPs as a colonization substrate. Furthermore, exposure to MPs and their leachates enhanced bacterial bioluminescence, with pristine and UV-aged MPs exhibiting maximum increases of 60% and 70%, respectively. Chemical analysis of MP leachates revealed a time- and concentration-dependent release of dissolved organic matter and metal ions, such as calcium (Ca^{2+}) and zinc (Zn^{2+}). A maximum concentration of 20 mg L⁻¹ Ca^{2+} was observed in leachates from 340 particles L⁻¹ of UV-aged MPs after 18-d release. Pearson correlation analysis confirmed that the stimulation of bacterial bioluminescence was primarily associated with the release of Ca^{2+} from MPs. In behavioral assays, *S. nematoptera* demonstrated a feeding preference for glowing MPs colonized by *P. phosphoreum* over non-glowing MPs, indicating that the bioluminescent properties of *P. phosphoreum* increased the visibility and attractiveness of the MPs to the fish. These findings suggest that interactions between MPs and bioluminescent bacteria could facilitate the ingestion of MPs by visually oriented predators, thereby increasing the potential for MP bioaccumulation and trophic transfer within marine food webs.

1.09.P-Mo073 Effects of Environmental Microplastic on Wild Wharf Roach (*Ligia exotica*) as Plastic Detritus Consumer

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Very few studies have clarified the toxic effects and translocation of environmental microplastics (MPs) pollution in marine resident species. To clarify the potential toxicity and gut microbial community variation on coastal resident species, wild wharf roaches (*Ligia exotica*) collected from Nae-do [as expanded polystyrene (EPS, commonly known as Styrofoam) uncontaminated area] and Maemul-do [as EPS-contaminated area] in south Korea. The MPs (>20 μ m) were detected highly in *L. exotica* from Maemul-do with average of 50.56 particles/individual. Especially, the concentration of Hexabromocyclododecanes, have been detected highly in *L. exotica* from Maemul-do (630.86 $\pm$ 587.21 ng/g l.w.). The results of comparative transcriptomic analysis revealed significant alteration of metabolic processes, mitotic cell cycle regulation, and immune system functions (cutoff: $q < 0.05$) in *L. exotica* from Maemul-do. In addition, the total operational taxonomic units of *Ligia* gut microbiome were higher in Nae-do than those of *Ligia* from Maemul-do. Alpha diversity showed a higher richness in *Ligia* from Nae-do. The relative abundance of phyla, families, and genera were different between *Ligia* gut microbiome from Nae-do and Maemul-do. These findings suggest that the diversity of the gut microbiome is host-specific, but the different abundances of bacterial groups may depend on diet, including plastic pollution. This work was supported by a grant Risk assessment to prepare standards for protecting marine ecosystem

1.09.P-Mo074 Effects of Poly(Methyl)Methacrylate (PMMA) Nanoplastics in Shore Crab *Carcinus maenas*: A Behavioural and Biochemical Assessment

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Nanoplastics (NP), plastic particles in the nanoscale, may pose a significant threat to oceans and marine life due to their ability to penetrate biological membranes. These particles may disperse in the water column, eventually reaching sediments where they can interact with benthic organisms.

Poly(Methyl)Methacrylate (PMMA), is a polymer with a high commercial importance, with applications in several human activities including biomedicine. Despite its increased use, there is limited knowledge regarding the effects of small particles of this polymer. Thus, this study aimed to assess the effects of PMMA NPs, on behavioural (locomotion), and biochemical endpoints (neurotransmission, antioxidant defenses and oxidative damage) of the epibenthic crab *Carcinus maenas*, a key species in the benthic community, with important roles in the marine food web and the nutrient cycle. The crabs were individually exposed, via water, to a concentration range (5-320 µg/L) of PMMA NPs (~513 nm), for 10 days. Results showed that crab locomotion was significantly affected by PMMA NPs exposure, with the crabs from the highest concentrations spending more time moving, but slower than control organisms. Effects on locomotion were associated with a decrease of muscle cholinesterase activity. In gills, an increase of glutathione s-transferase (GSTs) activity was observed for all PMMA NPs tested concentrations whereas superoxide dismutase (SOD) activity only increased for 20 µg PMMA NPs/L. In hepatopancreas, increase of SOD activity was observed after exposure to 5, 20, and 80 µg PMMA NPs/L, whereas GSTs activity decreased at 5 µg PMMA NPs/L. Both tissues revealed oxidative damage, with the increase of protein carbonylation (20, 80 and 320 µg PMMA NPs/L in gills and 5, 20 and 80 µg PMMA NPs/L in hepatopancreas) and lipid peroxidation levels (80 µg PMMA NPs/L in gills and 20, 80 µg PMMA NPs/L in hepatopancreas), with hepatopancreas as the tissue more affected. Overall, suggest that PMMA NPs may have potential impacts not only at individual, but more importantly, at a population level.

1.09.P-Mo075 Microplastics in Sentinel Species (Mussels, Polychaetes and Shrimps): Accumulation, Depuration and Tissue Localization in Experimental Set Ups and in the Environment

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Microplastics (MPs) in the marine environment have been identified as a growing hazard to marine biota. The aim of this study is to clarify the fate of MPs in different environmental compartments and their interaction with biota through microcosm experiments and a one-year biomonitoring program. MP depuration dynamics and accumulation patterns were assessed in target tissues of different sentinel species *Mytilus galloprovincialis* mussel, *Hediste diversicolor* polychaete, and *Palaemon serratus* shrimp, inhabiting the water column, the sediment and the interface between both. Organisms were exposed to different sized polystyrene MPs (1, 5, 10 µm) at different concentrations (103-105 particles/mL) up to 5 days, and left depurating 7 and 14 days. The field study was carried out during one year in the Biosphere Reserve of Urdabai (Basque Country) where water, sediment and biota were sampled seasonally. All samples were then subjected to quantitative (alkaline digestion and filtration) and qualitative (cryosectioning) examination under fluorescence microscope, and MPs were analyzed by Raman image spectroscopy. After microcosm experiments, all species accumulated MPs spiked in the water column and sediment irrespective of their habitat, proving the occurrence of MP vertical transference between environmental compartments. The accumulation in tissues was MP dose and size-dependent. Regarding depuration, mussels were able to remove a large amount of MPs, with a significant reduction for small particles. However, in polychaetes the accumulation of MPs increased significantly compared to exposure. For shrimps, no discernible reduction in MPs occurred after depuration. MPs were found mainly in the lumen and epithelia of digestive tract of mussels, in the hindgut lumen of polychaetes, and in the lumen of digestive tract of shrimps. Regarding biomonitoring, mussels and polychaetes did not show seasonal accumulation of MPs neither differences among sites. Transparent PET fibers were the predominant MPs found in both organisms. This study enable to get deeper knowledge on the occurrence and fate of MPs in marine ecosystems. Funded: Basque Government (IT1743-22, IT- 1446-22) and MICINN (PID2020-118685RB-I00).

1.09.P-Mo076 Transcriptomic and Metabolomic Responses of Juvenile Rockfish (*Sebastes schlegeli*) to Fiber-Shaped Polyethylene Terephthalate Microplastics

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Microplastics (MPs) are potentially harmful factors that are widely distributed in the aquatic environment. However, the molecular mechanisms underlying the toxicity of fiber-shaped MPs remain uncertain depending on size and type. Fiber-shaped MPs (Sizes: 200-400 μm , 3000 μm) made of polyethylene terephthalate (fPET) were exposed to juvenile rockfish (*Sebastes schlegeli*) at four concentrations (0.2, 2, 20, and 200 mg/L). And the toxic effects were assessed by cytotoxicity and oxidative stress, and the toxic mechanism were identified using transcriptomic and metabolomics analysis in rockfish exposed to microplastics for 20 days. The growth rate of rockfish decreased but activity of apoptosis and phagocytosis of leukocytes were increased after exposure of fPET. The enzyme activities of glutathione S-transferases and superoxide dismutase increased in the high-concentration MPs exposure group. The liver differential expression genes (DEGs) of rockfish exposed to fiber MPs of size 200-400 μm were related to lipid metabolic process, cellular component organization, cell differentiation, and response to stress. The DEGs of the liver of rockfish exposed to fiber MPs of size 3000 μm were related to steroid metabolic process, developmental growth, and cell differentiation. Metabolite analysis showed abundant arachidonic acid metabolism, biosynthesis of unsaturated fatty acids, and linoleic acid metabolism, which belong to lipid metabolic processes. These results might be show the fPET exposure related to process of the antioxidant system and homeostasis maintenance in juvenile rockfish. These results will be helpful in understanding the toxic effects and mechanisms in juvenile fish exposed to fPET.

1.09.P-Mo077 Plastic Debris in Mussels: Molecular Responses and Role in Okadaic Acid Uptake and Toxicity

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(1)Parana Federal University, Brazil, (2)CIIMAR, Portugal, (3)Federal University of Parana, Brazil Plastic pollution poses a significant threat to marine ecosystems, particularly through the smaller fractions, namely microplastics (MPs) and nanoplastics (NPs). In addition to the toxicity associated with their direct exposure, plastic debris can also adsorb harmful biotoxins produced by microalgae. Microalgal cells can interact with or incorporate smaller plastics, potentially facilitating the transfer of these biotoxins into other marine organisms. Therefore, this study investigates the interactions between plastic debris and marine microalgae, evaluating their harmful effects on bivalve organisms from a trophic transfer perspective. Laboratory experiments were conducted to examine the transfer of NPs (0.1 or 1 μm) to mussels via microalgae. Additionally, the role of plastic debris ($\sim 5 \mu\text{m}$) in adsorbing the lipophilic okadaic acid (OA) toxin and serving as vectors for its transfer was assessed. Using confocal microscopy, flow cytometry, HPLC, and omic approaches, we analyzed the NPs accumulation and alterations induced by its exposure, as well as the accumulation and effects of the adsorbed biotoxin in the digestive glands (DG) and other tissues (OT) of mussels. We anticipate that microalgae will transfer particles of varying sizes differently, leading to significant proteomic changes in mussels. Furthermore, preliminary results indicate that after three days of exposure, significantly higher concentrations of OA were detected in the DG, increasing from $1.81 \pm 0.21 \text{ ng g}^{-1}$ on day 2 to $12.84 \pm 4.50 \text{ ng g}^{-1}$ by day 14 in mussels treated with MPs adsorbed with OA. While alterations in gene expression and differentially expressed proteins (DEPs) are still under analysis, our findings highlight the potential for OA transfer by $5 \mu\text{m}$ plastic particles and their associated toxicity. This work emphasizes the risks posed by MPs and NPs to trophic chain stability and safety, not only due to their direct action on aquatic organisms but also by highlighting the role of microalgae in inadvertently facilitating the transfer of harmful particles to higher trophic level organisms. This work was funded by the International Atomic Energy Agency (IAEA) via research contract PHYTOPLASTOX (RC #23548) and by the Coordination of Superior Level Staff Improvement (CAPES, Brazil) via a PhD scholarship granted to E.P. This work was also supported by the Portuguese Foundation for the Science and Technology (FCT) through the project NanoPlanet 2022.02340.PTDC, and UIDB/04423/2020 and UIDP/04423/2020 contracts. M.J.A. and A.C. also acknowledge the FCT funding for the Scientific Employment Stimulus Program (2023.06491.CEECIND and CEECIND/03767/2018, respectively).

1.09.P-Mo078 Harmonizing Data for Dietary Microplastic Exposure: Insights into Seafood and Age-Specific Risks

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Over recent decades, the rapid increase in plastic production has led to heightened concerns about the pervasive presence of microplastics (MPs) in the environment. Defined as particles smaller than 5 mm, MPs are increasingly detected in various food categories, raising questions about human exposure and associated health risks. This study aims to evaluate dietary exposure to MPs, with a specific focus on seafood, and to assess the contribution of various food groups to total MP exposure. To address this challenge, we reviewed existing data on MP occurrence in bivalves and crustacea and analyzed findings from a previous study involving 218 food items purchased from mainstream food retailers. We employed exposure assessment techniques to estimate daily MP intake across three age groups (children, adolescents and adults), considering realistic and worst-case scenarios. Special attention was given to aligning and comparing data from diverse sources using standardized methodologies. Our results indicate that adults have an average MP exposure of up to 0.37 MP per kilogram of body weight per day under realistic conditions. In contrast, children experience the highest exposure, reaching 4.19 MP per kilogram of body weight per day in worst-case scenarios. These findings highlight significant variability in exposure levels based on age and dietary habits. This study highlights the critical need for harmonized methodologies to synthesize data from diverse sources, particularly in evaluating seafood as a major contributor to dietary microplastic (MP) exposure. MPs in food may act as vectors, potentially transporting contaminants and allergens, making them more significant for secondary risks than nanoplastics (NPs), which are more concerning due to their distinct toxicological profiles. By improving our understanding of MP ingestion through food, this research provides a foundation for future investigations into exposure sources, associated health risks, and strategies for mitigation. The research that yielded MP occurrence results, was funded by the Belgian Federal Public Service Health, Food Chain Safety and Environment through the contract RT 18/05 Plastic in Food. The complementary study and the literature review was done in the scope of IMPTOX project which was funded by The EU's H2020 framework program for research and innovation, grant number 965173 entitled An innovative analytical platform to investigate the effect and toxicity of micro and nanoplastics (MNPs) combined with environmental contaminants on the risk of allergic disease in pre-clinical and clinical studies

1.09.P-Mo079 Effects of Micro(nano)plastics on Amphibian Cell Lines

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Plastic materials released into the environment undergo abiotic and biotic processes that cause the formation of microplastics (MP 100 nm to 5 mm) and nanoplastics (NP < 100 nm). The increasing reports of the effects of these particles on biota have driven the search for more sustainable alternatives such as bioplastics. However, whether bio-based plastics are more eco-friendly than conventional fossil-based plastics remains uncertain. There is thus the need to increase the knowledge regarding the effects of fossil- and bio-based plastic particles, particularly for organisms under high anthropogenic stress, such as amphibians. Due to ethical constraints, direct testing on these organisms is limited, and in vitro cellular models using established cell lines appear as a potentially valuable approach to assess the risks of MP and NP. This study assessed the effects of MP and NP from two fossil-based polymers polymethylmethacrylate (PMMA) and polystyrene (PS) and one bio-based polymer, polylactic acid (PLA), on two amphibian cell lines: A6 (adult *Xenopus laevis* kidney epithelial cells) and XTC-2 (tadpole *Xenopus laevis* carcass fibroblast cells). The cells were exposed to PMMA NPs (0-400 mg/L) and PS and PLA MPs (0-200 mg/L) and cell viability was assessed using the MTT assay (after 72-hour exposure). The ability to generate reactive oxygen species was assessed using the DCFDA assay (after 3-hour exposure), and the ability to cause DNA damage was studied through an alkaline comet assay (after 24-hour exposure).

PMMA NPs had no significant impact on cell viability for both cell lines, despite the ability to increase ROS production. In contrast, PLA MPs significantly affected A6 cell viability in the highest concentration tested whereas PS MPs had no significant effect. The highest concentration of PLA and PS MPs decreased XTC-2 cell viability. Overall, the ability of plastic particles to affect DNA integrity was also shown.

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1.09.P-Mo080 Adsorption and Protection of Environmental DNA (eDNA) on Polymer and Silica Surfaces

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In environmental systems, microplastics (MPs) are found alongside environmental DNA (eDNA). eDNA is genetic material shed by organisms through decayed cells, serving as a valuable tool for non-invasive biodiversity assessments. Adsorbed onto particles, eDNA has been shown to be longer-lived than when in suspension, likely because of protection from enzymatic degradation. Consequently, measuring adsorbed eDNA on MPs could provide insights into plastics origin and environmental transport pathways. The interactions of eDNA with MPs in aquatic ecosystems is influenced by water chemistry and the surface properties of plastics, impacting the fate of genetic material. While MPs fate and transport have been studied extensively, the adsorption of eDNA onto polymer surfaces and its persistence remains less understood. Here we systematically studied eDNA adsorption onto polyethylene terephthalate (PET), polyethylene (PE), and silica (Si) surfaces, where the latter material was used as a proxy for comparison to natural particles. Using Quartz-Crystal Microbalance with Dissipation monitoring (QCM-D), the impact of varying ionic conditions that mimic freshwater and seawater environments was analyzed for adsorption rate and extent. The role of cations, particularly divalent ions such as Ca²⁺, in promoting eDNA adsorption through cation bridging was particularly important. PET exhibited the highest adsorption rates, followed by PE and Si. Adsorption of eDNA in seawater led to thicker and more rigid adlayers compared to freshwater conditions. However, eDNA adlayers were susceptible to enzymatic degradation and/or replacement by DNase in both freshwater and seawater, suggesting limited protection from decay. Consequently, the longevity and potential for transfer of material over time and distance is brought into question. This study provides insights into the adsorption mechanisms and stability of eDNA on MPs, advancing our understanding of how MPs influence the transport and persistence of genetic material, including antibiotic-resistant genes, in aquatic environments.

1.09.P-Mo081 Bioconcentration and Metabolism of 3H-6PPD quinone in Zebrafish (*Danio rerio*)

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6PPD is a commonly used antioxidant in the rubber industry. However, its oxidized form, 6PPD quinone, has recently been identified as toxic to fish. A quinone transformation product of 6PPD, known as 6PPD-Q, was isolated from TWP leachates and shown to be a major toxicant to coho salmon, as demonstrated through median lethal concentration (LC50) assays and retrospective analysis. In this study, we quantitatively measured the steady-state bioconcentration factor (BCF_{ss}) and metabolite concentrations of 6PPD quinone in zebrafish using radiolabeled compounds. Zebrafish were exposed to 1.0 and 10.0 ng/mL of 6PPD quinone for 24 days during the uptake phase, followed by a 7-day depuration period in the elimination phase. Based on total radioactivity residues (TRRs), the BCF_{ss} values were found to be 243 and 209 for the low- and high-concentration groups, respectively. During the elimination phase, a significant decrease of 85.6% and 94.7% in TRRs was observed after 5 days in the low- and high-concentration groups, respectively. Three metabolites (M-1, M-2, and M-3) were identified via Radio-HPLC. While M-1 and M-2 could not be fully characterized, M-3 was identified as monohydroxy 6PPD-Q through Q-TOF and MS/MS simulation.

1.09.P-Mo082 Histopathological Alterations in Adult Zebrafish Tissues Subchronically Exposed to Microplastics and Fipronil

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Microplastics (MPs; < 5 mm) contamination has emerged as a critical environmental issue due to their ubiquitous presence and negative impact on ecosystems and human health worldwide. MPs also have the capacity of adsorption of toxic pollutants in aquatic and soil environments. Fipronil (FP) is a highly effective insecticide widely used to control various arthropod pests, however it can also affect non-target species. Our objective was to evaluate the histopathological alterations in adult zebrafish tissues (gills, liver, and kidney) subchronically exposed (14 days) to a mixture of microplastics and FP. Zebrafish adults were exposed to several mixtures containing FP (0.01; 0.10; 1.0 µg/L) and a fixed concentration of polyethylene MP (100 mg/L). No mortality occurred during the whole experiment, nor in the control or in the exposed groups. The histological index of FP individually indicated significant reduction in circulatory and increase in progressive changes at 1.0 µg/L. However for the mixture histological index (progressive, regressive, circulatory, and total) showed no significant differences among the mixture treatments, indicating the MP presence may reduce FP toxicity. Regarding the liver, vacuolization increased in all fish after MP and FP exposure. Moreover, about 50-100% of the exposed fish presented necrosis in liver tissues. Significant changes in the mean nuclear diameter was shown among all treatments when compared to control. Lastly, for the kidney, no significant alterations were observed in the control groups.

However, after mixture exposure, degeneration of nephron tubules was observed in all organisms. With increased FP concentrations, fish exhibited increased alterations such as a reduction in Bowman's space, increased glomerular volume, and necrosis. The current findings indicate that the mixture of FP and MPs can induce considerable damage on adult zebrafish and may promote histopathological changes in gill, liver, and kidney tissues, demonstrating also that MP can reduce FP toxicity. This research was funded by São Paulo Research Foundation (FAPESP) grant number [2022/03094-5, 2024/10993-1, 2024/01418-3, 2023/09884-0 and 2023/15540-2] and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) grant number 153725/2024-9.

1.09.P-Mo083 Impacts of Microplastics and Glyphosate on the Microalgae *Arthrospira platensis*
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Microplastics (MPs, particles < 5 mm) have become a pressing environmental concern with multifaceted implications for aquatic life and ecosystem health. Despite the widespread presence of MPs in aquatic ecosystems is now well-established, the knowledge about the potential toxic effects in lower trophic levels, such as microalgae, is still largely unknown. The present study aims to evaluate the potential toxic effects of polyethylene terephthalate (PET) and polyamide (POLY), isolated or combined with the pesticide glyphosate (GLY), in *Arthrospira platensis*. For this, *A. platensis* were cultured in Zarrouks medium and exposed to a control, GLY (3 µg/L), PET (0.5 and 1 mg/L), POLY (0.5 and 1 mg/L) and the respective mixtures of each MPs with GLY (GLY+PET0.5; GLY+PET1; GLY+POLY0.5; GLY+POLY1), during 12 days. The optical density was monitored at 565 nm by UV spectrophotometer. At the end of the exposure, the photosynthetic pigment content (chlorophyll a, chlorophyll b, and carotenoids), phytochemicals (total phenols, flavonoids, and ortho-diphenols), antioxidant (ABTS + and DPPH) and enzymatic (oxidative stress and detoxification biomarkers) activity were determined. Microalgae growth was affected at 4 days of exposure, suffering a significant increase in the group exposed to GLY+POLY1. Nevertheless, no significant effects were observed on the biomass and the photosynthetic pigment contents. These results indicate that MPs or GLY alone seem to have little effect on the growth and chlorophylls of *A. platensis*. In contrast, it was observed modulation of the detoxification mechanisms, with glutathione-S-transferase being significantly increased in microalgae exposed to POLY0.5, and carboxylesterase activity being inhibited in the PET1 and GLY+PET mixture groups. The glutathione reductase activity and reduced glutathione (GSH) also showed a significant decrease in the PET1 and POLY1 exposed groups, respectively, indicating a higher consumption of GSH. Also, exposure to GLY alone or combined with PET or POLY increased total phenols, while GLY alone decreased flavonoid levels. The present results showed that microalgae interact with MPs, which can have serious consequences for food webs, food security, and ecological health. MPs, single and combined with GLY, affected both the detoxification mechanisms and non-enzymatic defense, including the shikimate pathway, in *A. platensis*, highlighting that more research in this field is urgently needed. This work is supported by National Funds by FCT - Portuguese Foundation for Science and Technology, under the project UIDB/04033/2020.

1.09.P-Mo084 Visual Evidence of Multigenerational Transfer of Nanoplastics in Pea Plants
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Plants are primary producers in soil ecosystems, and micro- and nanoplastics that entered into plants can be transferred throughout the soil ecosystem. Although studies on the nanoplastic uptake for plants are continuously reported, there are limited studies on generational transfer of nanoplastics. Thus, this study aimed to determine the generational transfer of nanoplastics on pea plants. We exposed nanoplastics (Polystyrene, 200 nm) to the first generation of pea plants (F0) in soil, and the second (F1) and third (F2) generations were replanted to clean soil. We found that nanoplastics were transferred to the grandchildren of peas exposed to nanoplastics, as well as daughter peas. In addition, transmission electron microscope analysis confirmed that nanoplastics entered both the intercellular and intracellular spaces of F2 peas. These findings suggest that once absorbed into plants, nanoplastics can be persistently transferred across generations, highlighting the potential for transfer to animals and humans. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (RS-2024-00355254).

1.09.P-Mo085 Size matters less? Exploring the Cross-species Intricacies of Nanoplastics Toxicity from Zebrafish to Chicken

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Plastic pollution, particularly in the form of microplastics (MPs) and nanoplastics (NPs), is an escalating environmental concern due to their potential toxicity and ability to enter organisms through various pathways. While NPs are typically considered more toxic than MPs due to their ease of tissue penetration, the size-dependent toxicity and mechanisms of NPs across different species remain insufficiently understood. This study investigates the toxicity of polystyrene nanoplastics (PS-NPs) of 50 nm, 200 nm, and 1000 nm in two model species: zebrafish larvae (aquatic) and chicken embryos (terrestrial). The primary challenge addressed by this work is the inconsistent toxic effects and toxicity levels of NPs, which vary based on particle size and species. Understanding these effects at multiple organismal levels is crucial. We hypothesize that NP toxicity varies by both size and species, with distinct mechanisms of action for aquatic and terrestrial organisms, even at similar developmental stages. Zebrafish and chicken embryos were exposed to the same PS-NPs (0, 0.1, 1, and 10 ppm) during comparable developmental windows. Toxicity was comprehensively assessed through behavioral assays, biochemical markers, RNA sequencing, IPA analysis, and 3D imaging. Results revealed species-specific responses: zebrafish exhibited hypoactivity and neurotoxic effects, particularly with smaller NPs (50 nm), while chicken embryos displayed more pronounced morphological effects with larger particles (200 nm and 1000 nm). IPA analysis identified similar hepatic and cardiovascular dysfunctions and diseases across species after PS-NPs exposures. These findings challenge the assumption that smaller particles are always more toxic. We emphasize the need for size- and species-specific considerations in environmental risk assessments, advancing our understanding of nanoplastic toxicity across ecosystems. This study was supported by Danmarks Frie Forskningsfond (0165-00056B) and Novo Nordisk Foundation (NNF18SA0032928).

1.09.P-Mo086 Microplastics Reduce Endocytosis of Earthworm Immune Cells

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Although the effects of microplastics (MPs) have been consistently studied, lack of research investigating the response of immune cells in soil invertebrates against MPs has been reported. The purpose of this study is to evaluate the effects of MPs on soil invertebrates at both individual and cellular levels. Earthworm *Eisenia andrei* and polystyrene microplastics (PS MPs) were selected as model organism and MPs, respectively. The in vitro assay for 24 hours and soil bioassay for 14 days were carried out. The in vitro assay revealed an increase in MP internalization by earthworm amoebocytes, followed by inhibited endocytosis in earthworm coelomocytes. Similarly, results from the soil bioassay indicated that MPs soil pollution suppressed coelomocyte endocytic activity and caused mitochondrial damage in the digestive tract. The findings from the present study provide evidence of environmental immunotoxicity of MPs in soil ecosystem. Acknowledgement-This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2021R1C1C2012628, RS-2023-00207834).

1.09.P-Mo087 Effects of Environmental Microplastics on Soil Microarthropods Survival, Behaviour and Biodiversity

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Microplastics (MP, size <1mm) are pervasive in terrestrial ecosystems, found not only in soils affected by various anthropogenic activities, such as urban settings, but also in remote areas due to long-range atmospheric transport and deposition. The widespread presence of MP in soils raises concern as they may negatively impact soil health and functions, as well as impair soil organisms throughout their life cycles. This study focuses on MP released by the weathering of Plastic Pavers for Parking (P3) in urban environments, aiming to: (i) assess the effects of P3-MP on collembolan species (*Folsomia candida*, *Parisotoma notabilis*, *Mesaphorura macrochaeta*); (ii) evaluate differences in springtails behaviour in the presence of MP; (iii) investigate the potential relationship between soil microarthropods community and MP abundance in contaminated sites using the Soil Biological Quality (QBS-ar) index. To reach the first goals, environmentally relevant MP were generated in the lab through abrasion of large HDPE fragments, collected from P3 in an urban area of Northern Italy. MP were analyzed via Scanning electron microscope (SEM) and Raman spectroscopy to ensure lack of microbial colonization and polymer degradation, respectively. Such MP were then used in bench-scale chronic (28 days, MP tested range: 0-10-100-1000mg/kg) and avoidance tests (48hours: MP tested concentration: 0-1000 mg/kg) with springtails, following OECD 232 and ISO 17512-2 protocols. As far as *F. candida*, a concentration-dependent increase in springtails mortality was observed after 28 days of exposure to MP. Compared to the control

group, which showed an average mortality rate of $17 \pm 5\%$ (mean $\pm$ SD), an increase up to $58 \pm 13\%$ in springtails mortality was recorded at 1000mg/kg. MP also altered the behaviour of the collembolans, with an avoidance rate of $34 \pm 13\%$ in the treated group, compared to the control group (avoidance rate of $3 \pm 4\%$). Furthermore, a field study was carried out to assess the impact of MP released from the P3 on the soil community. To this aim, soil samples were collected at increasing distances from the MP source and both MP and microarthropods extracted to determine abundance and composition. Preliminary QBS-ar index results suggest a positive correlation between the distance from the contaminated site (i.e., HDPE-P3) and soil microarthropod diversity. Our findings contribute to the understanding of MP levels and impact assessment in urban ecosystems under real exposure scenarios.

1.09.P-Mo088 Unveiling Microplastic and Metal Pollution in Giant Armadillos (*Priodontes maximus*)

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The Giant Armadillo (*Priodontes maximus*), the largest armadillo species, is classified as Vulnerable by the IUCN. In Brazil, *P. maximus* inhabits various ecosystems, including forests, savannas, and grasslands. The Rio Doce basin, located in the Brazilian Atlantic Forest, one of the world s primary biodiversity hotspots, has been recognized as a critical conservation area for this species. However, in 2015, the region experienced Brazil s largest environmental disaster when a mining dam ruptured, releasing approx imately 60 million m³ of tailings predominantly composed of iron and containing high levels of toxic metals such as manganese, aluminum, chromium, lead, and arsenic, which have posed a lasting contamination threat to the local ecosystem. Extensive research has documented that tailings from such mining disasters are toxic to biodiversity, affecting various species through bioaccumulation and habitat degradation for example. In parallel, the widespread contamination by microplastics has raised concerns about its impacts on wildlife health, as microplastics can cause a series of negative consequences for the exposed organisms. However, the presence of this pollutant and its effects have been studied only to a limited extent in large mammals. In this study, we investigated the presence of toxic metals and microplastics in feces samples collected from *P. maximus* within two regions of the Rio Doce basin. Microplastics were found in all analyzed samples, displaying different shapes, sizes, colors, and polymeric compositions, revealing widespread contamination within the habitat of this vulnerable species. Additionally, high concentrations of toxic metals were detected across the samples, suggesting significant exposure of these animals to pollutants from the 2015 disaster. These findings highlight the extent of environmental pollution in critical conservation areas and its impact on vulnerable species such as *P. maximus*. The consistent detection of both microplastics and toxic metals in samples of the Giant Armadillo underscores the susceptibility of this species to these contaminants. Our study is the first report of the occurrence of microplastic in armadillo feces.

1.09.P-Mo089 Biodistribution of Polystyrene Nanoplastics in Mice: Advancing Analytical Techniques Using Metal-doped Plastics

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Contamination of plastic particles in both environmental and biological systems has prompted concerns regarding their potential for negative impacts. As humans are increasingly exposed to microplastics (MPs)- and nanoplastics (NPs), there are potential adverse health effects related to uptake and chronic exposure by such particles, but they remain unclear, in part because of analytical challenges which exist to detect trace concentrations. Various studies have reported that NPs can cross biological barriers and are taken up into cells, where they can cause stress reactions and induce immune responses. To overcome analytical challenges when assessing NPs in biological samples in laboratory settings, we propose a workflow combining three complimentary methods, namely inductively coupled plasma mass spectrometry (ICP-MS), X-ray fluorescence imaging (XFI), and imaging mass cytometry (IMC) for detection of model metal (palladium)-doped NPs (PdNPs). While ICP-MS can quantify metals and metalloids in trace concentrations in different matrices, XFI and IMC provide additional spatial resolution, even down to the cellular level. This workflow was used to quantify the temporal distribution and accumulation of PdNPs in model mouse systems, covering different biological conditions, dosages, and

time frames. Short-term exposure to high particle doses showed a clear excretion pathway from the gastrointestinal tract into feces while continuous oral intake over longer time periods led to tissue accumulation of PdNPs, including in distal organs. By assessing plastic particles in complex matrices with these analytical tools, we were able to better quantify and understand uptake and translocation mechanisms of particles down to the nanoscale in mice.

1.09.P-Mo090 Detection of Model Micro And Nano Plastics in Living Cells With Synchrotron Techniques

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The distribution of nanoplastics (NPs) in the environment is increasingly becoming a cause of concern for human health. The small size of these particles makes them not only prone to accumulate in the tissues of various organs but also enables them to enter cells and act as carriers of external materials and microbes. To allow strategies for investigating cellular interactions and toxicity mechanisms in different in vitro systems, we propose model nano plastics (NPs) made of the two among the most environmentally relevant polymers (polypropylene and polyvinylchloride) and properly labelled with cadmium-selenide quantum dots. The presence of these semiconductor nanocrystals gives them unique properties that enable light fluorescence imaging. Additionally, it allows for the use of synchrotron radiation X-ray imaging combined with X-ray fluorescence micro-spectroscopy (TwinMic at Elettra synchrotron) to detect NPs with high resolution by mapping the distribution of the composing element Se. The human umbilical vein endothelial cell line (HUVECs) was selected for this study as an in-vitro model, providing a relevant and reproducible system for investigating vascular toxicity and the broader effects of MNPs on human health. They were grown in a complete culture medium onto the appropriate substrates for X-ray analyses, finally exposed to relevant concentrations (20- 50 µg/ml) of QDs-PP-, PVC- NPs (50-350 nm sized) Cryo-nano-tomography was used to investigate NPs uptake and internalisation in a three-dimensional visualisation by exploiting the different absorption contrasts of the labelled NPs compared to the cell matrix. Afterwards, the Se QDs component distribution was successfully used to track, by XRF microscopy, the NPs across the cells, while endogenous elements such as Na, Mg, C and O were monitored to investigate potential biochemical changes attributed to NPs toxicity. Together with the 3D reconstruction provided by Crio-nano-tomography, two-dimensional absorption and phase contrast imaging were important to reveal morphological changes, such as the accumulation of vesicles, in response to PP-NPs. PE- and PVC- NPs exposure. In conclusion, when using proper NPs models, high-resolution 3D and 2D imaging, combined with XRF analyses, is a powerful tool for biological studies aimed at deepening our understanding of the toxicological mechanisms of pollutants on human immune response and fertility. Funding: Ministry of Health, Italy by IRCCS-Burlo Garofolo (2022 RC38/23).

1.09.P-Mo091 Effects of Polyethylene (PE) and Polyvinyl Chloride (PVC) Plastic Particles on Isolated Human Erythrocytes

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Micro- and nanoplastic particles have been shown to be environmental contaminants with potential health risks. Once plastic particles are ingested or inhaled, nanoplastics and small microplastics can enter the bloodstream and affect human erythrocytes, altering their morphology, aggregation behavior and membrane integrity. It has been previously demonstrated that model particles of polystyrene (PS) can cause a deleterious effect on erythrocytes. The aim of the present study was to produce realistic polyethylene (PE) and polyvinyl chloride (PVC) particles and to investigate their effect in vitro on erythrocytes in concentrations analogous to those found in human blood. For this purpose, PE and PVC particles were initially produced via grinding and subsequent filtration to a diameter between 700 and 400 nm. Subsequently, erythrocytes were isolated from human blood samples and incubated for up to 20 hours with 1, 10 or 50 µg/mL of PE or PVC sub-microplastic particles, as well as with 100 nm and 1 µm PS model particles. Following incubation under cell culture conditions, an analysis was conducted to investigate the impact of alterations in cell number, interaction with plastic particles and resulting changes in cell morphology. The results indicate that the addition of plastic particles did not affect erythrocyte count in low concentrations. However, significant morphological alterations were observed in the erythrocytes exposed to sub-micro PE and PVC particles, as well as to micro- and nano-sized PS model particles. These findings suggest that even at physiological concentrations, plastic particle exposure may impair the ability of erythrocytes to pass through blood vessels efficiently. In future studies, the

incorporation of plasma proteins and the use of a broader range of particle sizes, will provide a more comprehensive understanding of the dynamic interactions between micro- and nanoplastics and erythrocytes under more realistic in vivo conditions.

1.09.P-Mo092 Detection of Microplastics in the Human Penis

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Recent investigations have shed light on the prevalence of microplastics (MPs) in human tissues, such as stool samples, lung, placenta, and cardiac tissue, sparking a crucial discourse about their health repercussions [1 6]. For humans, the primary pathways for MP exposure are ingestion, inhalation, and dermal contact. Annual intake estimates range from 39,000 to 52,000 MPs per person, predominantly through ingestion [7, 8]. These particles can penetrate the body directly via the atmosphere, drinking water, and sea salt, or indirectly through the food chain. Inhalation presents another route, with average daily intake estimated at 272 MPs, which can lead to lung inflammation and other respiratory complications [9]. Notably, recent preliminary studies indicate that MPs can impact fertility and sperm quality, thus threatening reproductive success [10]. This can include live sperm count reduction and morphological abnormalities [11]. While direct evidence linking MPs in penile tissue to erectile dysfunction (ED) is lacking, existing research on environmental factors affecting penile health and erectile function offers valuable insights. Studies such as Sorkhi et al. (2022) on microvascular perfusion, Jung et al. (2014) on neurogenic structures, and works by Jaeger & Walker (2016) and Sopko, Hannan, & Bivalacqua (2014), highlight the multifactorial nature of ED, potentially exacerbated by environmental pollutants such as MPs [12 15]. In this study, we employed laser direct infrared (LDIR) analysis to detect the existence of MPs in penile tissue of individuals undergoing inflatable penile prosthesis (IPP) placement for the treatment of ED. The detection of MPs in penile tissue would create a new avenue for the environmental impact on sexual health, prompting inquiries about the sources, pathways, and potential ramifications of MPs exposure on erectile function.

1.09.P-Mo093 Impact of Micro- and Nanoplastics on Human Monocytes: A Focus on Autophagy, Lysosomal Integrity, and Mitochondrial Function

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Micro- and nanoplastics (MNPs) are an emerging environmental concern due to their ubiquity and potential impacts on human health. Humans are exposed to MNPs via ingestion, inhalation, and skin contact. Recent studies have shown that MNPs can induce cellular stress by altering the autophagy-lysosomal system, meanwhile triggering inflammatory and cytotoxic responses, ultimately leading to cell death. Indeed, MNP-induced alterations at the level of the autophagy-lysosomal functions pose significant risks to cellular health by exacerbating chronic inflammation and cellular stress, thus contributing to the development of diseases like neurodegeneration, fibrosis, and metabolic disorders. However, little is known about the mechanisms of MNP internalization and their effects on human cells, especially upon intracellular organelle dynamics and functions. Here, we evaluated the impact of MNPs of different sizes, such as 0.5, 1, 5 μm , on freshly isolated human monocytes, focusing on autophagy, lysosomes, and mitochondrial function. Transmission electron microscopy (TEM) was used to examine ultrastructural MNP localization and organelles. Gene and protein expression related to the autophagy-lysosomal pathway were also analyzed using RT-qPCR and western blot, respectively. Alterations in mitochondrial functions were assessed via oxygraphy. Our results showed that MNPs are internalized by monocytes through endocytosis and accumulate within lysosomes featuring a multilamellar morphology, which went along with massive autophagosome accumulation. TEM imaging also revealed alterations in mitochondrial morphology, while oxygraphy indicated changes in the respiratory chain. Consistent with these observations, genes and proteins associated with autophagy (e.g., LC3) and lysosome formation (e.g., LAMP1) were upregulated. Notably, the evaluation of the autophagy process was performed with MNPs concentrations calculated with IC50 curve. In conclusion, this study provides novel insights into the

cellular and molecular mechanisms of MNP internalization in human monocytes and highlights the potential damage caused by MNP exposure to immune cells. These findings contribute to understanding the long-term health risks associated with MNPs and underscore the need for further research on their impact on human health. This research was supported by the PNRR_PRIN23CFENI_01 grant.

1.09.P-Mo094 Investigating the Production, Reproducibility, and Particle Characteristics of Fluorescence-Labeled Micro-Nano-Plastic Reference Material and Their Possible Cell Uptake

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Plastics play a crucial role in many industries due to their numerous advantageous properties, particularly in product packaging. Although designed for durability, overuse and inadequate recycling have led to significant environmental accumulation. When exposed to natural conditions, plastic fragments into smaller particles, including micro- and nanosized fragments, which accumulate in the environment and can re-enter the human system. Despite extensive research, the health effects of nanoplastics remain unclear. As the amount of micro- and nanoplastics (MNPs) in the environment is expected to rise, understanding their potential health impact is crucial. A major challenge in this area is the lack of standardized reference materials. Many studies focus on plastics with varying sizes, chemical structures, and properties, with much research centered around polystyrene (PS), leaving gaps in knowledge about other plastics. Two alternative widely-used plastics, polyethylene terephthalate (PET) and polypropylene (PP), are more interesting for cell interaction studies, due to their differing hydrophobicity and density. From this perspective detectability is an important consideration, and developing fluorescence-labeled materials could significantly improve detection. This project aimed to produce well-characterized PET and PP particles labelled with the fluorescent polymer poly(9,9-dioctylfluorene-alt-benzothiadiazole) (F8BT) to assess their cellular uptake and cytotoxicity in lung cell models. The particles were prepared by co-precipitating PET or PP with varying F8BT concentrations (0.8%, 3%, 5%), their size was measured via laser diffraction, and their fluorescence properties were analyzed. An in-silico model, RiskGone in vitro dosimetry, was used to calculate a theoretical delivered dose, based on which an administered dose was chosen for the uptake experiment conducted with CALU-3 or U937 cells. A desired size distribution (<1 µm) was reproducibly achieved for PET with 5% F8BT and PP with 3% F8BT. The limit of detection (LOD) and limit of quantification (LOQ) improved with higher dye loading. For PET, the LOD was 0.86 µg/mL and the LOQ was 2.61 µg/mL, while for PP, the LOD was 3.40 µg/mL and the LOQ was 10.30 µg/mL. Preliminary uptake experiments showed that PET was taken up by cells to some extent, while PP showed no measurable uptake, possibly due to insufficient sedimentation onto the cell monolayer.

1.09.P-Mo095 In Vitro Effects Of Micro- And Nanoplastics On The Human Immune System

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The growing prevalence of micro- and nanoplastics (MNPs) in the environment raised concerns regarding their potential impact on human health. Chronic exposure to MNPs has been linked to inflammatory responses and immune system impairment, increasing the risk of chronic and autoimmune diseases. Previous in vitro and in vivo studies have demonstrated that MNPs can alter activity and gene expression immune cells. However, the exact mechanisms by which MNPs and aged MNPs modulate the immune system remain largely unexplored. The aim of this project is to analyze the in vitro effects of MNPs on human immune cells, focusing on the immunological pathways involved. Specifically, we evaluated MNPs cellular alterations in cell proliferation and viability, changes in gene expression and inflammatory responses, and their impact on phagocytosis. The effects of different concentrations and sizes of MNPs were evaluated on primary human monocytes. Monocyte internalization of MNPs was assessed using confocal microscopy and flow cytometry. Flow cytometry was also used to monitor the expression of immune system activation markers, and gene expression was analyzed via qPCR, focusing on genes related to antigen presentation, inflammasome, and inflammation. The same experimental procedures were applied to determine the effects of photoaged MNPs by ultraviolet (UV) exposure. Monocytes rapidly internalized MNPs, causing reduced cell viability and increased apoptosis, showing a size and dose-response relationship with immune activation. We detected increased production of some inflammatory cytokines, such as TNF α and IL8. Additionally, MNPs influenced monocyte differentiation, increasing the expression of activation markers like CD80 and HLA-DRII in classical monocytes. Gene expression analysis revealed a reduction in HLA-A expression, which may influence antigen presentation. Furthermore, levels of NLRP3 and CASP1 were reduced, suggesting decreased inflammasome activation. Notably, preliminary data also indicate that UV-aged MNPs induce a more pronounced inflammatory response compared to untreated MNPs, suggesting that photoaging may amplify the immunotoxic effects of MNPs. The findings suggest that MNPs significantly affect the innate immune response, with

detrimental effects on cellular viability, inflammation, and immune function. However, more studies are needed to assess the long-term risks of MNPs exposure, focusing on in vivo models simulating chronic exposure conditions. This research was supported by the PNRR_PRIN23CFENI_01 grant.

1.09.P-Mo096 Impact of Microwave-Treated Polypropylene Microplastics on Caco-2 Cells

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Plastics are subject to various forms of deterioration, including photo, mechanical, and chemical degradation. These processes break plastics down into smaller particles known as microplastics. Due to their ubiquitous presence, microplastics can come into contact with human cells, particularly through ingestion. This is especially relevant for food containers made of polypropylene (PP) one of the most widely produced plastics in Europe which is commonly used for food storage and microwave heating. Studies have shown that PP particles can be released from food containers after microwave treatment or prolonged storage. While PP has been detected in the human colon, its potential effects on human health remain unclear. To investigate these effects, the Caco-2 cell line is frequently used in in vitro experiments as a model of the intestinal epithelial barrier. Our study aimed to investigate the impact of PP particles microwave-treated on Caco-2 cells, focusing on cell viability, membrane damage, and oxidative stress. Caco-2 cells were exposed to PP particles at concentration 200µg/ml and subjected to different microwave treatment cycles (3 minutes cycle repeated 1, 5, 10 times - and a 30 minutes continuous at high power (1000 W)), for 24 and 48 hours. Cell viability was assessed using the MTT assay, cytotoxicity/membrane damage were evaluated through LDH assay, and oxidative stress levels were quantified using CellRox. The findings revealed a rise between 13 35% in cell viability across all PP microwave-treated after 24 hours of exposure. However, a subsequent decline was observed after 48 hours in all conditions, with PP Pristine and PP microwave 30 minutes continuous with the most significant decrease rates of 23% and 22%, respectively. No membrane damage was detected in Caco-2 cells following PP microwave-treated exposure. Furthermore, oxidative stress levels surged by a minimum of 74% across all experimental conditions after 24 hours, persisting at elevated levels compared to negative control samples after 48 hours. These findings align with previous studies on other plastics, highlighting oxidative stress as a potential mechanism affecting cell viability and supporting the implications of our research. Our findings contribute to understanding of the biological effects of plastic exposure, urging for continued investigation into the broader health implications of plastic usage.

1.09.P-Mo097 Intracellular Fate of Polymer Nanoparticle Systems Monitored Through mid-Infrared Photothermal Microscopy

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Perfluorocarbon (PFC)-encapsulating poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) are promising 19F MRI probes, particularly for drug delivery and theranostic applications. In this study they served as model system for investigating the intracellular breakdown processes of polymer-based nanoparticle system. These processes are not yet fully understood, thus it is crucial to observe how cells process, degrade, and metabolize them after endocytosis. This understanding is essential for optimizing safety and efficacy in imaging and therapeutic applications and has implications for other polymer particle types. For investigating the intracellular fate of particle components, Mid-Infrared Photothermal (MIP) Microscopy, also known as Optical Photothermal Infrared (OPTIR) spectroscopy, was utilized. This spectroscopy technique enables precise chemical analysis by delivering component-specific spectral data. This study focused on two particle systems, nanoparticles (NPs) and nanocapsules (NCs), which were incubated with RAW macrophages for 2 and 24 hours. Spectral comparisons of cells, along with NPs, NCs, and their isolated single components, allowed the tracking of particle breakdown. Additionally, chemical maps were generated at specific wavenumbers to quantify particles within a cell. The use of OPTIR spectroscopy setup facilitates precise chemical imaging of intracellular samples by allowing spatial differentiation between particles and cellular components. Based on the results of these chemical images, points of interest inside the cells are selected and full spectral analysis is performed. This analysis revealed a significant reduction in PLGA signals after 24h inside the cells, indicative for PLGA degradation within the cells. This study highlights the potential of OPTIR spectroscopy to provide label-free and detailed insights into intracellular fate mechanisms.

1.09.P-Mo098 Valorizing Fungal Biomass from Microplastic Bioremediation

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Microplastics are ubiquitous contaminants and, recently, much focus has been placed on the potential use of bio-based methods for their removal and mitigation within environmental matrices. Among these biotechnological strategies, fungi have played a predominant role, owing to their resilience and ease of use, coupled to reduced or inexistent health risks. While the efficacy of fungal bio remediation in microplastic removal has been demonstrated, the subsequent fate of the fungal biomass remains largely unexplored. Herein, we explore the potential antioxidant and antimicrobial activities of extracts obtained from *Penicillium brevicompactum* used for biologically mediated removal of low-density polyethylene (LDPE). Bioremediation assays showed that the fungus was able to remove approximately 40% of the LDPE microplastics in 21 days. The generated biomass was then subjected to high-pressure extraction and the obtained extracts were tested for antioxidant and antibacterial activities. Antioxidant and antibacterial tests showed bioactivity of these extracts, and these activities may be further explored and potentiated by the inclusion of bioactive compounds derived from additional types of fungi, as currently under evaluation.

By repurposing the fungal biomass, waste generation may be minimized and reduction of the environmental impact associated with conventional waste disposal methods may be achieved.

Furthermore, bioactive compounds extracted from the fungal biomass could serve as valuable raw materials for various industries, such as cosmetics, pharmaceuticals, and food additives.

While the limited bioactivity of the extracts in this study may not immediately translate into commercial applications, it underscores the immense potential of fungal biomass as a valuable resource. Further research and technological advances are needed to fully realize the benefits of this innovative approach.

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1.09.P-Mo099 The Power of Electron Microscopy in Plastic Particle-Cell Interaction Studies

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Electron microscopy (EM) provides valuable insights into the interactions between particle and cells and enables detailed visualization of their effects at the cellular and subcellular levels. Scanning electron microscopy (SEM) is essential for studying cell morphology, while transmission electron microscopy (TEM) visualizes intracellular organelles and the potential ultrastructural changes caused by particle uptake. In addition, SEM coupled with focused ion beam (FIB/SEM), where an ion beam mills the sample and an electron beam images it, provides both surface and subcellular analyses in a 3D mode. The aim of our work was to evaluate the suitability and usefulness of EM on plastic particle-cell interaction studies. We used SEM, TEM and FIB-SEM to investigate the interactions between polystyrene nanoparticles and A549 cells (a surfactant-producing alveolar type II cell model). We first grew A549 cells under standard submerged conditions and treated cells with plastic particles at increasing concentrations. We then created a 3D culture model as a more realistic test system combining transwells and an air-liquid interface (ALI) setup. The SEM analysis provided valuable 3D information about the surface of the cell model highlighting the interactions of the particles with the microvilli. TEM examination showed that under standard submerged culture conditions, the particles are mainly localized in the endosomes and occasionally in the few lamellar bodies (LBs) of cells, possibly affecting the endolysosomal compartment. TEM analysis also reveals the presence of enlarged ER cisternae and secretory lysosomes at the cell surface, as well as a reduction in LBs and mitochondria-associated ER membranes (involved in surfactant production) and an increase in the number of endosomes in treated ALI samples. The FIB/SEM study provided an information of the intracellular presence of particles and LBs, while the other structures could not be identified. Here we provide evidence that electron microscopy provides valuable information on the particle-cell interactions, which could be a basis for assessing additional stress related biomarkers. We also point out the significant advantage of FIB/SEM, which makes possible in situ selection of cells to be

milled to inspect their interior. In addition, FIB/SEM on dry, and not in plastic embedded samples, allows to discriminate intracellular components with different physical properties despite similar chemical composition. This work was funded by Horizon 2020 project PlasticsFatE.

1.09.P-Mo100 A Comprehensive Analysis of Plastic Pollution in Greenhouse Agricultural Environments: The Case of Almería (Spain)

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The widespread presence of plastic waste in ecosystems has become a critical global concern due to its pervasiveness and associated environmental and health risks. This plastic pollution arises from various economic sectors, including intensive agricultural activities. The Almería region in south-eastern Spain hosts one of the largest areas of plastic greenhouses in the world. This study aimed to trace the fate, distribution and accumulation of mismanaged plastic waste from these greenhouses within the surrounding environment of Almería (Spain), as well as to investigate the potential of these plastics to act as carriers of plant pathogens once they reach the ecosystems. We identified, characterized and quantified plastic waste dispersed across both abiotic and biotic environmental matrices surrounding the greenhouses, extending to coastal marine areas where plastics may potentially end up, including a protected nature reserve. Additionally, we analysed the abundance of plant pathogens associated with the presence of plastics in the biotic matrices. Abiotic matrices included greenhouse and outdoor air, lagoons water, seawater, and coastal marine soils; while biotic matrices involved various wildlife populations including invertebrates, fish, rabbits, hares and foxes, in which we analysed the presence of plant pathogens attached to plastics by examining whole individuals or their faeces. Our findings revealed the presence of plastic waste in all examined environmental compartments, suggesting that a portion of this waste likely originates from the greenhouses. Furthermore, plastics were found across every animal taxon examined. Plastic waste was also present in the nature reserve, exhibiting concentrations that were not consistently lower than those in non-protected areas. This research represents the first comprehensive investigation of plastic pollution linked to greenhouse agriculture in the region of Almería (Spain). The authors acknowledge the Ministry of Science and Innovation of Spain for supporting this research through grant PLEC2021-007693 and RYC2021-034953-I.

1.09.P-Mo101 Trends in Study Quality and Reporting in Microplastics Research

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Interventions through published recommendations or perspective articles are a common practice to advance scientific research and create more reliable output from experimental studies. In 2020, de Ruijter et al. defined quality reporting criteria for microplastic effect studies (with extensions added by Mehinto et al. in 2022). Since then, the number of publications on microplastics effects on organisms and human health-related endpoints has increased exponentially and it is unknown, whether de Ruijter s intervention resulted in effects on study quality in the microplastics community. The Toxicity of Microplastics Explorer (ToMEx) 2.0 database contains quality scores for almost 300 microplastic ecotoxicity studies published through January 2023, including technical quality scores and scores aimed at defining adequacy for use in environmental risk assessments. We used these data to assess whether microplastics study

quality has changed over time and how the quality of reporting is associated with the taxonomic group of the test organism and journal impact metrics. We found that most studies reported basic technical aspects like the name of the test species, the polymer type of the particles, particle shape and size and characteristics of the experimental design (e.g., exposure duration, sample size), but information involving more complicated procedures like, for instance, measurements of background contamination, analytical investigation of the chemical purity of the particles and the verification of exposure were missing more often. In addition, we found that many studies in ToMEx 2.0 did not comply with necessary risk assessment criteria including the testing of aged or biofouled particles or following a proper dose-response design with more than three concentration levels. We show that over the years and by January 2023, study quality according criteria from de Ruijter et al (2020) and Mehinto et al (2022) has not increased. Study quality however correlated slightly with journal impact factors. We further found that research on some taxa achieved higher than average quality scores (Mollusca, Annelida, Crustacea, Hexapoda/Insects) whereas studies on fish received scores lower than average. We emphasize the importance of high quality reporting and test designs and discuss how better reporting practices and knowledge about requirements for risk assessments can contribute to create publications with higher impact on regulation in the future. M.M.M. is grateful for funding from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) SFB 1357 391977956.

1.09.P-Mo102 Paint: A Ubiquitous Yet Disregarded Piece of the Microplastics Puzzle

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Microplastics are widespread pollutants. Microplastics generated from the wear and tear of paints and coatings have recently been modeled to be a large source of microplastics to the environment. Yet, studies focused on microplastics broadly frequently overlook paint microplastics. In this article, we systematically reviewed the primary literature (turning up 53 relevant articles) on paint microplastic sources, identification methods, environmental concentrations, and toxicity to model organisms. Examples of sources of paint microplastics include paints from buildings and murals, crafts and hobbies, cars and roads, marine boats and structures, and industrial systems like pipes, sewers, and other infrastructure. Paint microplastics have been quantified in several marine samples from Europe and, to a lesser extent, East Asia. Reported concentrations of paint microplastics are up to 290,000 particles per kilogram of sediments, with the greatest concentration reported near a graffiti wall. Out of the toxicity studies testing paint microplastics, there have been 68 tested effects in total across all endpoints and organisms and 17 quantified lethal concentration 50% doses (ranging from 0.001 to 20g/L). Of the tested effects, 45 observed endpoint values in the paint treatment were significantly different from the control (66%) most of which were tests using antifouling paints. Overall, the number of studies on paint microplastics is small, limiting a holistic understanding of microplastics. Based on our synthesis of the state of the science on paint microplastics, we suggest a research agenda moving forward informed by research gaps. We thank Environment and Climate Change Canada (ECCC; Y.C., C.M.R.) and the Liber Ero Postdoctoral Fellowship (Z.T.D.) for supporting this research.

1.09.P-Mo103 Sniping the Hotspots: Identification, Analysis and Elimination of Microplastic by Microflotation from Sedimentation Tanks

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From the past few years, Micro- and Nanoplastic (MNP) has been identified as one of the most emerging threats to human health and eco systems. After decades of promises for circular economy, we are standing at 9% global recycling rate whilst plastic production didn't peak, yet. Facing the increased MP pollution issues in an economic way, we developed a combined and scalable approach comprising the three following steps: 1. Identification of the MP hotspot by rapid measurement methods, 2. profound analysis accounting for size and polymer distribution of items and masses, and 3. an eliminating microflotation step, which is adjustable for various aqueous environments and incoming MP concentrations. Hydrophobic interactions between particle and microbubble interfaces are the driving forces for separation. Since these interactions are not highly selective for MP only, there is a strong need for focusing on appropriate hotspots enabling higher removal efficiencies and preventing unintended discharge of common biological materials. In this context, tire and road wear particles (TRWP) are considered as the most abundant MP class and, hence, sedimentation tanks are focused as time variant hotspots directly connected to polluting infrastructures. In this study, state-of-the-art analysis techniques are used to qualify MP removal efficiencies for prototype systems under model water and environmental conditions in a semi-technical lab scale and representatively selected sedimentation tanks, accordingly.

1.09.P-Mo104 Development of Methods For Materials and Analytical Techniques of Metal Embedded Nanoplastics and their Application to Environmental Behavior Evaluation

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Plastics that enter the environment undergo a weathering process through chemical (e.g., photodegradation) or physical (e.g., abrasion) pathways, which promotes fragmentation into smaller particles and the generation of microplastics. In particular, microplastics smaller than 1000 nm are classified as nanoplastics, which are more prevalent in the environment and have high mobility, making them important carriers of other pollutants. Recently, studies have increased showing that nanoplastics can be absorbed by flora and fauna, posing serious threats to ecosystems and human health. This has highlighted the need for research to understand the environmental behavior and characteristics of nanoplastics. Conventional nanomaterial analysis methods (e.g., SEM, TEM, DLS) face limitations in the quantitative analysis of nanoplastics due to difficulties in distinguishing them from background particles and calculating particle number concentrations. This study aims to develop materials for investigating the environmental behavior of nanoplastics and to establish quantitative analysis techniques based on these materials. We synthesized nanoplastic materials embedded with rare metals using general-purpose resins such as polystyrene (PS), polyvinyl chloride (PVC), and the hydrophilic polymer polyvinyl pyrrolidone (PVP). This study proposes methods for characterizing these materials, classifying nanoparticles by size, and calculating particle number concentrations and size distributions of nanoplastics based on the size and concentration of metal ions using single particle inductively coupled plasma mass spectrometry (spICP-MS). The metal-embedded nanoplastic materials offer the advantage of being easily distinguishable from background particles in complex matrices due to the element specificity of spICP-MS, allowing for precise quantification of the behavior of various nanoplastic mixtures under identical experimental conditions. Additionally, this method can be applied to assess and predict the behavior of nanoplastics (e.g., weathering, aggregation, mobility) under various laboratory-based environmental conditions.

1.09.P-Mo105 Detailed Characterization of Polymers and Microplastics by KMD Plots of Complex MS Spectra

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For the analysis of microplastics, a detailed material characterization can be essential to identify its origin, fate and potential toxicity. Since most of these plastic samples comprise of a complex mixture of ingredients including several types of polymers and co-polymers plus various end groups, the mass spectral analysis can be quite complicated. Here, we introduce MALDI-TOF-MS in combination with Kendrick Mass Defect (KMD) plots as a powerful and convenient tool for assessing information from microplastics. The benefit of the KMD plot is its ability to simplify the interpretation of complex polymer spectra. By plotting polymer signals on a two-dimensional plane based on their KMD values, a clear linear trend emerges with the addition of repeat units, facilitating easier interpretation even for mixed samples. This unique feature makes the KMD plot invaluable for unraveling the composition and structure of intricate mixtures in plastic material. An autoflex maX MALDI-TOF (Bruker) was used in positive reflector mode for data acquisition of model plastic samples. Samples were dissolved in THF at a concentration of 10 mg/ml and mixed with matrix as well as NaTFA for an increased ionization efficiency. The acquired mass spectral data were processed by the flexAnalysis software for peak picking and external calibration with the Bruker fleXstandard polymers, followed by data analysis in Polytools 2.0 (Bruker) for KMD plot display and homopolymer assignment. In addition, Polymerix 3.01 (Sierra Analytics, Modesto, CA) was used for copolymer spectra interpretation. Data will be shown from various complex plastic and polymer samples. With the extremely high complexity of the spectra, a manual spectrum interpretation becomes very time consuming or even impossible to perform. In the KMD plot, the various polymers show up in horizontal or diagonal lines or grid-like structures revealing the polymer subunit including the presence of co-polymers, possible oxygenation and various end groups. PolyTools generates a resulting table summarizing the information from the KMD plot. For those distributions in the KMD plot which were assigned to copolymers, a further investigation was carried out by Polymerix. E.g., samples revealed propylene glycol, esters of one propylene glycol with phthalic acid (PGPA) as monomer B.

1.09.P-Mo106 Accurate High Throughput Microplastics Characterization on Aluminum-Coated Filter Using the Agilent 8700 Laser Direct Infrared (LDIR) Chemical Imaging System

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Indeed, microplastic pollution has become a significant environmental concern due to its potential impacts on ecosystems and human health. The Agilent Laser Direct Infrared (LDIR) chemical imaging system represents an innovative approach to imaging and spectral analysis, particularly in the context of microplastic identification and other applications that require chemical characterization of materials. Depending on the level of suspended solids presents, ASTM D8333 describes the steps required for microplastics sample preparation. Regardless of analysis technique used, samples with various levels of microplastics eventually require a filtration step. At present, the Agilent 8700 LDIR microplastics sample introduction can be achieved directly on gold-coated filters. To minimize the cost associated with gold-coated filters while maintaining easy and efficient sample introduction method, accurate microplastics characterization with the 8700 LDIR can be performed on aluminum-coated polyester filters. This study outlines the utilization of the Agilent 8700 LDIR in achieving accurate microplastics characterization on aluminum-coated filters in terms of particles detection, particle count repeatability, particle size accuracy and identification of common microplastics.

1.09.P-Mo107 Micro- and Nanoplastics — A Wish List for Reliable Determination of the Risks for Humans

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The assessment of risks to human health from micro- and nanoplastics (MNPs) and their associated chemicals is hampered by significant uncertainties and data gaps regarding human exposure, biological fate and potential health effects. There is also a notable lack of validated test MNPs, natural particles to serve as reference controls, and lack of harmonised toxicological assessment methods specifically designed for the unique properties of MNPs. These limitations contribute to data scarcity and further complicate exposure and hazard assessment for MNP and their associated chemicals. The European research cluster CUSP (<https://cusp-research.eu/>) aims to fill these gaps by providing reliable data and suggests approaches for assessing human exposure and health risks associated with MNPs and their associated chemicals, including contaminants. As part of this initiative, five projects address the health risks of MNPs from different perspectives, focusing on different exposure pathways and health effects. In particular, the PlasticsFatE project (<https://www.plasticsfate.eu/>) structures the data requirements and develops strategies to overcome both general and material-specific barriers to MNP in hazard and risk assessment. Based on safety research on engineered nanomaterials, which has shown that it is impossible to experimentally test all MNP variants and their chemical mixtures, CUSP emphasises the need for pragmatic approaches. These approaches aim to effectively utilize knowledge from nanomaterial research, emphasize the reuse of generated data on fate and effects of MNPs, as well as identify and address data and knowledge gaps along with uncertainties, while considering the specific properties of MNPs and test approaches. This poster provides a structured overview of the current challenges in the risk assessment of micro- and nanoplastics and introduces the first step of categorising the different types of obstacles. Specific strategies to overcome these different types of challenges will be presented. This work was funded by the European Union's Horizon2020 Research and Innovation Programme, under the Grant Agreement number 965367 (PlasticsFatE).

1.09.P-Mo108 Detection of Microplastics in Amniotic Fluid and Placenta in Preeclampsia and Healthy Full-Term Pregnant Women without Underlying Conditions

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Microplastics (MPs) are now ubiquitous in the environment and can enter the human body through various exposure routes. Concerns have risen around their impact on human health, especially in vulnerable populations such as pregnant women and their fetuses. Preeclampsia (PE), a pregnancy-specific

hypertensive disorder, is characterized by high blood pressure and organ dysfunction, often linked to placental vascular abnormalities and inflammatory responses. Although studies have confirmed MPs in placental and amniotic fluid samples, data linking MPs to perinatal complications, such as PE, remain scarce. Given that MPs may induce inflammation and oxidative stress, their presence could exacerbate PE-related placental dysfunction, posing risks to both maternal and fetal health. This study aims to systematically detect and analyze MPs in amniotic fluid and placental samples from pregnancies complicated by PE and compare these findings with MPs detected in samples from healthy, full-term cesarean deliveries in women without PE or other underlying conditions. Under appropriate IRB approval, informed consent was obtained from pregnant women scheduled for cesarean delivery at Seoul National University Bundang Hospital. During the procedure, amniotic fluid and placental samples were collected, adhering to stringent protocols to prevent plastic contamination. Cotton, glass, and metal instruments were exclusively used for sample handling and storage. MPs were detected using μ -Raman spectroscopy following extensive pre-processing to eliminate organic materials. Blank control tests were conducted to ensure that air, collection instruments, and pre-processing reagents were free of plastic contamination. We analyzed MP particle size, type, and concentration, comparing findings between PE and healthy, full-term control groups. Additionally, inflammatory markers in amniotic fluid (e.g., CRP, interleukins, MMPs) were measured via ELISA to assess differences in inflammatory responses between the two groups.

1.10.A Exploring the Complex Dynamics and Ecotoxicological Impacts of Micro- and Nanoplastics in Aquatic Systems

1.10.A.T-01 Exploring Vegetation Complexity as a Driver of Microplastic Accumulation in Coastal Marshes

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Coastal vegetated wetlands, such as mangrove forests, seagrasses, and saltmarshes, are thought to act as traps for both macro- and microplastic pollution. While microplastics occurrence in coastal vegetated sediments is well documented, there is conflicting evidence on whether the presence of vegetation enhances microplastics trapping relative to unvegetated sites. There are many potential underlying drivers of microplastic entrapment that have yet to be explored. Through a combination of laboratory flume experiments and field observations, we investigated three factors influencing microplastic trapping in vegetated wetlands: vegetation structure, species diversity, and microplastic type. In a controlled flume experiment we tested the effects of grassy and branched forms of vegetation structure and microplastics that differed in shape, size, and polymer type on microplastic trapping efficiency. Following this, we collected sediment samples from four levels of species diversity across a saltmarsh (Blakeney National Nature Reserve, United Kingdom) to explore how microplastic accumulation patterns change from the laboratory to the field. Quadrats (N = 20; n = 5) were randomly stratified in areas with no vegetation, monospecific grass (*Spartina anglica*), monospecific branched (*Atriplex portucaloides*), and diverse (>3 species) sites. From the laboratory experiment we observed that the presence of vegetation did not affect the number of microplastics trapped but did affect location of deposition. Microplastic shape, rather than polymer, was the dominant factor in determining whether microplastics were retained in the sediment or adhered to the vegetation canopy. We predict that our field observations will reveal similar patterns to the flume experiment in addition to highlighting interacting effects from both biological and physical processes. The outcome of this work will enrich our understanding of coastal vegetation as a microplastics sink and inform where hotspots of microplastic accumulation are most likely to occur within a biogenic canopy. By increasing our understanding of microplastics pollution within highly valued coastal habitats, we can aid protection, restoration, and potential clean-up efforts.

1.10.A.T-02 Leaching Potential of Weathered Ocean Plastics: Insights from Cross Sectional Metals Analysis and Mapping

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Plastic pollution poses an environmental threat, with millions of tons of waste entering the oceans annually. Once in the ocean, plastics undergo various aging processes due to their prolonged exposure to harsh marine environments including UV radiation from sunlight, wave and wind stress, mechanical abrasion, thermal oxidation, and biodegradation. Through these weathering processes, their chemical and physical properties are altered and hazardous metals natively in the plastics can be released. Alternatively, inorganic substances in the water can sorb onto the plastic surface. While analytical techniques such as

FT-IR and Raman spectroscopy are commonly used to assess polymer composition, they do not allow for the detection of inorganic substances, such as metals, within the plastic matrix. This study aims to address metal distribution within pristine and ocean-weathered plastic items recovered from marine environment to better understand potential pollutant uptake and release. Laser Ablation Inductively Coupled Plasma Time-of-Flight Mass Spectrometry (LA-ICP-TOFMS) allows to perform elemental mapping with high spatial resolution. By sectioning plastic samples and scanning their cross-sections, it provides a deeper understanding of metal distributions, patterns of accumulation, and leaching tendencies. Our analysis identified distinct metal distribution patterns, including: (1) surface enrichment of biofilm-associated elements such as Na and Mg, (2) uniform distribution of some metals such as Al, Cu, and Zn, potentially used during manufacturing, (3) gradual surface-to-core increases in elements like Mg, Ca, I, suggesting adsorption, and (4) leaching of manufacturing-related elements like Sb. Correlation analyses further distinguished elements likely acquired during marine exposure from those originally incorporated into the plastic during manufacturing. This study demonstrates the potential of LA-ICP-TOFMS mapping for detailed profiling of weathered plastics, providing critical insights into pollutant dynamics in marine environments. These findings enhance our understanding of metal uptake and leaching in weathered plastics, contributing to the assessment of ecological risks and informing strategies for managing plastic pollution in marine ecosystems.

1.10.A.T-03 A High-Resolution Spatial Model to Predict the Distribution of Microplastics in European River Basins: ePLAS

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Microplastic pollution has emerged as a pressing environmental concern, particularly in aquatic ecosystems where rivers act as key conduits transporting these pollutants. While models for microplastic transport in river systems do exist, they are typically limited to smaller-scale environments or single catchments, and comprehensive, high-resolution models for extensive geographic regions like entire countries or continents remain scarce. This study utilizes the existing ePiE (Exposure to Pharmaceuticals in the Environment) framework, integrating it with multimedia box modeling to assess the fate of microplastics within the lacustrine and riverine systems at a high spatial resolution (~1x1 km). Our approach combines per capita emissions of microplastics with spatially detailed data on wastewater treatment plant (WWTP) locations and characteristics across Europe. This integration allows for precise predictions of microplastic concentrations at specific coordinates downstream of emission points. The model considers distinct physical properties of microplastics (size, shape, and density) and incorporates various fate processes such as advective transport, heteroaggregation, sedimentation-resuspension, burial, and bed load transport, thereby providing a comprehensive analysis of microplastic dynamics in water and sediment layers. Initial observations from a case study conducted in the Elbe River Basin suggest that the model can identify potential hotspots of microplastic accumulation, demonstrating its potential as a promising tool for developing strategies to mitigate the environmental impact of these contaminants.

1.10.A.T-04 Investigations of Fragmentation, Aggregation and Settling of Microplastic in Complex Riverine Environments

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Polymeric materials (PM), including microplastics (MP) and nanoplastics (NP), undergo complex transformation processes in riverine systems, altering their size, shape, density, and surface chemistry. These transformations, driven by factors like flow conditions, polymer properties, and environmental factors, are crucial for understanding MP behaviour and their ecotoxicological impacts. A holistic approach including fragmentation, aggregation and settling on PM is presented to improve the understanding of MP in complex aquatic environments. Fragmentation experiments using a Taber Abraser demonstrated that MP release rates vary by polymer type, with polystyrene exhibiting the highest rates and polyamide the lowest. Despite differences in release rates, particle size distributions were consistently bimodal, suggesting overlaying fragmentation mechanisms and a mechanical fragmentation threshold. Shore D hardness was identified as a key predictor of MP release, with insights to support the development of models that estimate MP release during bed load transport using sediment transport data. Aggregation and sedimentation experiments focused on low-density polyethylene (LDPE) particles modified by UV irradiation and biofouling. Surface modifications reduced hydrophobicity, increasing water affinity and enhancing particle aggregation and settling. Laboratory and environmental experiments, including field exposure studies in the Elbe River, revealed that modified LDPE particles are more likely to settle under specific conditions, despite their low density. These findings provide a framework to evaluate how laboratory results translate to real-world riverine processes. This research highlights the

importance of considering polymer-specific behaviours in MP monitoring strategies and transport models. By incorporating dynamic changes in particle size and shape, the study offers practical tools for assessing MP release and transformations in aquatic systems. Ultimately, the findings enhance the understanding of MP ecotoxicological impacts, supporting effective river management and pollution mitigation strategies.

1.10.A.T-05 Microplastic Fate and Transport in Rivers: A Flume Experiment

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Microplastic (MPs) pollution in aquatic environments has become a growing concern and understanding their fate in rivers is important to predict particle exposure as part of an environmental risk assessment. To date, a growing number of studies have focused on the interactions between MPs and sediments, while the sedimentation of MPs in such systems has hardly been investigated. Here we constructed an experimental flume setup designed to capture MPs dynamics in flowing water and predict their sedimentation probability based on tracked particle trajectories. We developed a low-cost, reproducible setup using a GoPro Hero 12 Black camera and developed Python code in-house to analyze videos. Using 3D printed sediment beds of varying grain sizes (plane, sand-sized, and gravel-sized), MPs fragments of five different polymer chemistries (PET, PA, PS, PE, PP) with densities ranging from 0.90 to 1.4 g/cm³ in three discrete size classes (ranging from 100 µm to 1000 µm) were assessed. Water flow velocities reached up to 0.2 m/s with a slope of up to 1%. MPs sedimentation velocities varied with polymer type, particle size and flow conditions, in agreement with established sedimentation theories. However, small particles, particularly under highly turbulent flow conditions with rougher sediment beds, remained in suspension regardless of polymer density. Finally, we found that the cavity traps observed were not sufficient to settle larger MPs with densities lower than water (e.g., PP) despite turbulences introduced from the sediment bed. In summary, our findings could be used in existing MPs fate and transport models to predict large-scale exposures in water bodies, which can improve environmental risk assessments.

1.10.B Exploring the Complex Dynamics and Ecotoxicological Impacts of Micro- and Nanoplastics in Aquatic Systems

1.10.B.T-01 New versus Naturally Aged Greenhouse Cover Films: Degradation and Micro- and Nanoplastics Characterization under Sunlight Exposure

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Micro- and nanoplastics generated by the degradation of plastic products can persist in the environment for prolonged periods, contributing to pollution and posing risks to wildlife and human health. Their presence has been documented across various ecosystems, including soil, and in food and beverages. Compared to macro- and mesoplastics, micro- and nanoplastics (MPLs and NPLs) exhibit distinct behaviors and physicochemical properties. The formation of smaller MPLs and NPLs via photodegradation has been demonstrated in earlier research, including studies quantifying NPLs derived from MPLs of polystyrene (PS), polypropylene (PP), and low-density polyethylene (LDPE) under UV exposure, as well as investigations into MPL formation from LDPE films with varying crystallinity. Additionally, Sorasan et al. identified NPLs emerging during the photoaging of LDPE in water and from marine plastic debris containing PE, PP, and PS, while Song et al. reported MPLs and NPLs in weathered expanded PS containers. Other studies have examined the release of MPLs and NPLs from diverse plastic items, such as tea bags, clothing during laundering, and disposable coffee cups. However, many of these investigations overlook the varying effects of aging on naturally aged (used) plastics compared to new ones. To gain an in-depth understanding of particle quantity and size distribution resulting from the photodegradation of greenhouse LDPE cover films, we analyzed both new and used LDPE films over six months using advanced nanotechnology methods. These cover films, originating from Almeria, Spain, where more than 30,000 hectares are devoted to greenhouses, underscore the importance of our work due to the typical six-month planting and harvesting cycle of most vegetables in this area. Raman spectroscopy, electron microscopy, and nanoparticle tracking analysis were utilized to characterize the chemical signatures, size and shape distributions, and concentrations of the plastic particles released following simulated sunlight exposure over this period. Our study showed that new and naturally aged greenhouse cover films release nanoplastics and exhibit different micro- and nanoplastic release behaviors. Importantly, our data highlight the importance of using multiple techniques to analyze the release of nanoplastics from the degradation of plastic products.

1.10.B.T-02 Unveiling the Leaching Dynamics of Hydrophobic Additives from UV-Weathered Plastics Using a Cosolvent Approach

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Plastics exposed to UV radiation undergo surface weathering, leading to physical degradation and chemical transformations that affect their environmental behaviour. This study focuses on the leaching dynamics of hydrophobic additives from UV-weathered custom-made polyethylene (PE) and polyvinyl chloride (PVC) materials with known polymer chemistry and additive compositions. While most studies simulate aquatic environments by submerging plastics during UV weathering, this work uniquely explores surface (dry) UV weathering followed by immersion in a cosolvent system to mimic environmental leaching scenarios. Custom plastics were exposed to UV light for up to 48 hours, during which significant visual changes were observed. PE materials exhibited varying degrees of discoloration, influenced by the type and concentration of additives such as UV stabilizers, antioxidants, and flame retardants. PVC samples showed pronounced degradation in the absence of stabilizers but remained visually stable when additives like Tinuvin P were present. These results highlight the protective roles of specific additives against UV-induced surface weathering. To study leaching behaviour of hydrophobic additives, a cosolvent system of acetonitrile and water was used, addressing challenges posed by extremely low solubility and slow leaching kinetics in pure water. Results indicated that leaching rates increased with higher acetonitrile concentrations. However, UV exposure effects on leaching were inconsistent, with lower additive concentrations observed in UV-exposed samples compared to dark controls. This discrepancy suggests formation of transformation products, or redistribution of surface additives, as supported by the US EPA's Chemical Transformation Simulator. By employing a cosolvent approach, this study condensed environmental leaching timescales into laboratory experiments, enabling accurate quantification of hydrophobic additives in pure water. These findings provide insights into the environmental fate of hydrophobic additives and highlight the complexity of additive leaching dynamics under UV exposure. Future research should focus on understanding transformation products and surface additive interactions to better predict environmental impacts.

1.10.B.T-03 Unveiling the Multi-Tier Effects of Commercial PVA-based Dishwasher Pods on *Danio rerio* Embryos: Insights on the Role of Additives

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Water-soluble polymers (WSPs) are synthetic polymers widely applied in industrial, medical, and consumer products. Despite their prevalence, WSPs are not regulated as plastics or chemical contaminants, leading to the lack of monitoring, production estimation, and circular economy action plans. This regulatory gap allows their uncontrolled release into aquatic ecosystems, raising considerable concerns about their potential risks to organisms and human health. Therefore, this study aims to evaluate the potential effects of polyvinyl alcohol (PVA), one of the most common WSPs, comparing the standard powder, and PVA-based dishwasher pods in both powder and liquid form, on the freshwater biological model *Danio rerio* embryos exposed for 120 hours post-fertilization (hpf) to the estimated concentration (0.1 mg/L) for the civil wastewater treatment plant of Milan-Nosedo (N. Italy). Detection of additives in the two types of dishwasher pods was performed using Proton Nuclear Magnetic Resonance (1H-NMR), while a multi-tier approach was employed to evaluate the effects across molecular, cellular, physiological, and organism levels, including proteomics and metabolomics analyses, genotoxicity (micronuclei formation, apoptosis and necrosis), reactive oxygen species (ROS) levels, acetylcholinesterase (AChE) activity, as well as mitochondrial respiration and glycolysis. Additionally, heart rate was measured as physiological endpoint, while at the organismic level various endpoints (e.g. distance moved, turn angle, and thigmotaxis) were assessed to evaluate the swimming behaviour of *D. rerio* embryos. The 1H-NMR spectrum of PVA standard compared to powder pods revealed only few differences, while many for liquid pods, suggesting the presence of several additives. Although the measurement and processing of some endpoints are still in progress, we observed a significant alteration ($p < 0.05$) for ROS, AChE, and heart rate of embryos exposed to powder pods compared to controls, contrary to those exposed to the PVA standard. These findings suggest that the additives present in commercial PVA-based products could be responsible for the observed toxicity, aligning with our previous studies. Further analyses are ongoing to better understand the ecotoxicological impact of these everyday PVA-based products on aquatic organisms.

1.10.B.T-04 In Vitro Toxicity of an Environmental Micro and Nanoplastic Mix Representative of Plastics Collected at Five Beaches of the Bay of Biscay

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Despite increasing micro and nanoplastic (MNP) research, limited information on toxicity of realistic environmental MNPs exists. This study aims to assess the toxicity of an environmental MNP mix representative of plastics collected in beaches of the south west part of the Bay of Biscay (BoB) on RTL-W1 cells. Plastics were collected following the OSPAR guideline once per season in 5 beaches of the Basque Country: 4 in Spain from January to October 2023 and 1 in France from July 2023 to April 2024. Polymer composition of each item was identified by FTIR and then items were micronized to <250 µm by polymer. MNPs were mixed keeping the polymer composition by weight to produce MNPs representative of each beach and season (winter, spring) and of the overall mix of all beaches and seasons (BoB-eMNPs). For toxicity assessment leachates (lch) and extracts (ext) were made by adding filtered distilled water, DMSO or methanol to MNPs at 10 g/L and agitating them (24h, 190 rpm, 18°C, darkness). The liquid phase was recovered by filtration (0.8 µm). In methanol ext, DMSO was added postfiltration (1:1) and methanol was evaporated with N₂. Cells were exposed to lch (0.01-100%) and ext (0.01-1%) for 24h for cytotoxicity evaluation. Then, cells were exposed to 20% (per beach and season) or 10% (BoB-eMNPs) and 1% lch and exts for 4h or 24h to assess ROS production and EROD activity, respectively. 11 polymers were identified in the items collected in the beaches. On a weight basis, a representative mix of plastics beached at the Bay of Biscay consists mainly of PE, PVC, PP and smaller amounts of PA, PS, PET and PUR. Per beach and season, all lch caused cytotoxicity at 40% dilutions and almost all ext at 1%. ROS increased significantly in cells exposed to all lch at 20%, while different responses were observed for 1% dilution of lch, DMSO and methanol ext. EROD activity was induced in cells exposed to almost all lch, but not to all ext. The lch of BoB-eMNPs showed the highest cytotoxicity, with less than 50% of cells surviving at 40% dilution and no cells surviving at 100% lch. Cytotoxicity of DMSO and methanol ext was similar in BoB-eMNPs as in beach and season MNP samples. ROS increased in cells exposed to 10% lch of BoB-eMNPs, while EROD activity was induced for all lch and ext of BoB-eMNPs. These results highlight the complexity of analyzing toxicity of environmental MNPs due to differences in polymer types and proportions, additives, sorbed pollutants and aging degree. Funded by Spanish MICIU (project FIERA), Basque Government (grant to consolidated group IT1743-22 and postdoctoral fellowship to NGS), and Euskampus (LTC AquEus).

1.10.B.T-05 Predicting Microplastic Toxicity for Aquatic Organisms Using Machine Learning

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Similar to Quantitative Structure-Activity Relationship (QSAR) models, which link the molecular structures of chemicals to toxic outcomes, models are needed to link MNP traits to their toxicities. We used the Toxicity of Microplastics Explorer (ToMEx) 2.0 database, which compiles data on MNP effects on aquatic species from the published literature. Two datasets were prepared: (i) a high quality dataset with data points filtered according to the quality criteria outlined by de Ruijter et al. 2020 (2,264 data points); (ii) a full dataset from which only data points with missing values (NAs) were removed (4,768 data points). We applied a 5-fold cross-validation scheme to train boosted regression trees on tasks to predict the toxicity. We compared the predictive performance of the two models and used Average Marginal Effects to gain insights into the relationships between toxic outcomes and MNP traits, experimental parameters, and species traits. Despite the high heterogeneity of our data, our models are able to predict the presence/absence of effects with almost 80% accuracy. This performance was the same for both datasets, suggesting that concentrating on high quality publications for data mining might be a valid measure to reduce data mining efforts. Based on averaged marginal effects, we found that for both models, increasing concentration and particle surface-area-to-volume ratio were associated with a higher likelihood of observing a toxic effect. In contrast, measures linked to increased particle size (particle surface area, length and volume) were associated with a lower likelihood of observing a toxic effect. Based on current data availability from published studies, it is unfortunately impossible to link toxic outcomes to specific MNP properties (i.e., beyond shape, density, and dimensions). The reason for this is

that MNP properties are often not measured and largely lack reporting (e.g., zeta potentials/surface charges are only rarely reported). We argue that standardized experimental setups could help increase comparability of results. Also, a stronger focus on characterizing MNP traits is needed to disentangle the specific effects of different MNP properties. By identifying key toxicological MNP properties, models similar to the ones we present here will be able to recognize less toxic combinations of microplastic traits, which can support reliable hazard and risk assessments, as well as the development of new, environmentally safer materials. This study was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) SFB 1357 391977956.

1.10.P Exploring the Complex Dynamics and Ecotoxicological Impacts of Micro- and Nanoplastics in Aquatic Systems

1.10.P-Tu058 Assessing the Plastic Removal Efficiency of Riverine Litter Collection and Prevention Solutions

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Rivers play a key role in the global plastic pollution crisis, by connecting sources of plastic litter from anthropogenic to natural environments. However, efforts to mitigate plastics in rivers have been scarce. In this work, we focus on solutions to reduce the current levels of pollution, which include technologies and actions designed to collect plastics already polluting rivers or to prevent them from entering waterways. Estimating the plastic removal efficiency of each solution is essential for evaluating its costs and benefits, enabling policymakers and stakeholders to advance with informed decision-making. Furthermore, it supports the development of customized and sustainable strategies to reduce plastic pollution. The assessment method should account for the diversity of solutions developed so far, but also the range of locations where they can be deployed (e.g., urban/rural river section, ports, water treatment facilities), and the spatial and temporal variability of the plastic pollution in rivers (e.g., due to tides, seasonality, hydroclimatic changes, and irregular pollution inputs). Due to this complexity, no harmonised method has been adopted to evaluate the efficiency and effectiveness of the solutions, hampering their comparison. In the INSPIRE project, we developed a new harmonized method to assess the efficiency of plastic removal solutions in the field. With it, we cover manual cleanups and ten technologies that are being deployed and tested in different river, urban water, and wastewater treatment plant locations in Europe (Danube, Douro, Kamni ka Bistrica, Rhine, Po, Scheldt). To do so, we revised existing methodologies and organised the assessment method in a modular approach that combines versatile protocols with pre-defined and qualified test materials that mimic the environmental plastics target by the solution. This combination enables us to carry out release-catch experiments to determine the percentage of plastic litter removed by the technology or action and its characteristics (e.g., plastic size range). This approach resulted from an effort between academia and industry to create improved guidelines for quantifying plastic removal efficiencies in a way that is both comparable and unbiased. It also ensures flexibility and adaptability to accommodate a wide range of solutions with diverse characteristics, that target different plastic sizes, and under distinct conditions. Innovative Solutions for Plastic Free European Rivers (INSPIRE) is funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Climate, Infrastructure and Environment Executive Agency (CINEA). Neither the European Union nor the granting authority can be held responsible for them. This project has received funding under grant agreement No 101112879 (INSPIRE).

1.10.P-Tu059 Evaluation of the Effectiveness of Flocculants in the Removal of Plastics in Wastewater Treatment Plants and Related Ecotoxicological Impacts

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The pervasive presence of plastics in the environment and their increasing release into natural ecosystems represent a significant concern for both human and environmental health. Wastewater treatment plants (WWTPs) are identified as a primary source for plastics to enter water courses. Although various studies have demonstrated the efficiency of certain polymer-based flocculants in removing plastics from wastewater in laboratory tests, their potential application in WWTPs remains underexplored, and numerous commercial polymer-based flocculants have not yet been tested. To address this gap, the aim of this study is the evaluation of the effectiveness of different commercial polymer-based flocculants, synthetic and natural, to decrease the plastic concentration in wastewaters. To ensure the applicability of these flocculants in real-world scenarios, wastewater samples will be collected from two distinct type of WWTPs (civil and industrial) to assess their effectiveness across different matrices and mixtures of plastics. The outcome will provide insights into the practical applications and potential environmental advantages of using these flocculants in WWTPs, contributing to the development of better strategies for plastic mitigation and promoting more sustainable treatment processes. To achieve this goal, given that flocculation is one of the final depuration processes in wastewater treatment, it is imperative to also evaluate the effects that flocculants could be in aquatic ecosystems, their interaction with plastics and the potential decrease of plastics ecotoxicity by using the freshwater crustacean *Daphnia magna*, one of the most used aquatic biological models. Specifically, the study will investigate the exposure of this model-organism to the most effective concentrations identified for each of the flocculants and plastics observed in the removal efficiency experiments. The investigation will span various levels of biological organization, from molecular to organismal. It will assess potential alterations in the proteome, as well as changes in behavior, reproduction, and feeding patterns. This comprehensive approach aims to provide a detailed understanding of how these treatments may influence aquatic organisms and ecosystem health.

1.10.P-Tu060 Modelling the Environmental Fate of Microplastics with UTOPIA, An Evaluative Unit World Model for Microplastics

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Plastic pollution is pervasive in the environment as a complex footprint of particles of different sizes, shapes, compositions and properties. Despite the efforts made over the last decades to understand microplastics' fate in the environment, uncertainties about their sources, transport, transformation processes, and microplastics properties are still prevalent. To advance our understanding of plastic pollution and its associated risks, knowledge integration and extrapolation is urgently needed. In this context, process-based mass-balance models offer a useful platform for synthesising knowledge about sources, processes and pathways, and estimate exposure concentrations of plastic pollution in all forms. Here we present UTOPIA, an open-source process-based mass-balance model, that addresses the plastic footprint challenge by simulating the fate of plastic particles across 17 environmental compartments, accounting for five size classes and four aggregation states. By solving a system of coupled mass-balance equations, UTOPIA estimates steady-state concentrations, persistence, and transport metrics such as travel distance for the five size classes and four aggregation states globally and per compartment. Integrated sensitivity and uncertainty analyses allow exploration of key factors influencing plastic fate. Accessible via a web-based interface, UTOPIA enables users to define emission scenarios, identify dominant reservoirs, and explore transport and transformation processes. The model serves as a reference framework for screening-level risk assessments, hypothesis generation, and identifying knowledge gaps in understanding microplastic pollution. The authors thank Cefic Long Range Research Initiative for their funding support of the project LRI ECO56 (UTOPIA). The authors acknowledge support from InfraVis for providing application expertise for visualization and web-interface development through the Swedish Research Council grant 2021-00181.

1.10.P-Tu061 Untangling the Influencing Factors Governing the Interaction Between Plastics and Metals: A Focus on Conventional and Compostable Plastics

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Plastics, the most pervasive pollutants in ecosystems, pose a significant environmental threat by serving as vectors of waterborne chemicals. These materials seem capable of adsorbing and releasing (trace) metals. Their adsorption onto plastics may result in concerning environmental impacts, posing interest in the alteration of their environmental fate. The sorption capacity of plastics is influenced by various factors, many of which remain poorly understood. To address this critical knowledge gap, clarifying how polymer type, additive content, and ultraviolet (UV) degradation affect this phenomenon is essential. Our study

aims to investigate the adsorption likelihood of copper (Cu) and lead (Pb) on plastics in aquatic environments. We selected two polymers, namely a conventional polymer (low density polyethylene) and a compostable polymer (polybutylene adipate terephthalate, since this type is commonly used as environmentally friendly alternative). For both types, pristine pellets - ideally without any additive - and end-use plastic materials were considered. All these samples were both analyzed as they were (i.e., pristine) and after a degradation process induced by UV radiation. These samples were compared regarding their physicochemical properties (i.e., surface morphology, functional groups) and their sorption affinity towards Cu and Pb (after adsorption kinetics experiments). The adsorption process was also validated by assessing the metal content before and after the sorption experiments. The results of adsorption experiments highlight the key role of additives in plastics as a primary factor influencing adsorption capacity. In fact, end-use plastics show significant metal adsorption, unlike pristine pellets, which show none. Regardless of the other factors, the UV degradation can increase the adsorption capacity, as well as the presence of the compostable polymer. The nature of the interaction was also assessed through kinetic models. Both pseudo-first order and pseudo-second order models explained adsorption, suggesting a complex, multi-step process. Our study provides valuable insights into the behavior of plastics with heavy metals, emphasizing the roles of UV degradation and chemical composition. These results highlight the critical influence of chemical composition on plastic behavior in the environment, as well as the uncertain behavior of compostable polymers.

1.10.P-Tu062 Bridging Laboratory and Nature: Exploring the Adsorption Behaviours on True-to-Life Microplastics

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Although microplastics are widely recognised as a modern environmental threat, current studies predominantly employ polymeric models, i.e., pristine spherical microplastic particles (microbeads). These model materials enable the unraveling of modes of action, mechanisms, and other insights, but they share little to no analogies with microplastics found in nature. Therefore, there is an urgent need to create a material that better reflects the real characteristics of naturally formed microplastics, bridging the boundaries between laboratory parameters and the rules of nature. To address this problem, we investigated the broad-spectrum of adsorption capabilities of a novel, true-to-life type of test microplastics obtained by employing different fragmentation techniques, namely ultracentrifugal milling and mixer milling, using commercially available plastic products. In particular, we investigated the adsorbome profile through the biofilm formation with various biotic components, the absorption of metal cations, i.e., Ni²⁺ and Pb²⁺, and also the exposure to other organic pollutants such as perfluoroalkyl substances. Biofilm formation on these microplastics alters their physicochemical properties, potentially enhancing pollutant uptake. These results suggest that true-to-life microplastics provide more reliable assessments of environmental risks associated with microplastics. Furthermore, our preliminary findings demonstrate that true-to-life microplastic exhibit different adsorption behaviours depending on the polymer type, metal cation, and/or organic pollutant investigated. The study underscores the importance of employing environmentally relevant microplastic test materials to better understand their interactions with pollutants and biota, thereby improving the consistency of environmental risk assessments and informing mitigation strategies. This contribution is based upon work from COST Action CA20101 Plastics monitoring detectiOn RemedlaTion recovery PRIORITY, supported by COST (European Cooperation in Science and Technology, www.cost.eu). S.F. acknowledges the Italian Ministry of University and Research (Research program Research Projects of National Relevance, PRIN 2022) and the project PLASTACTS 202293AX2L, CUP D53D23009050001.

1.10.P-Tu063 Factors Impacting Resuspension of Microplastics from Rivers: Analysis of Burial and Shielding Effects of Sediment Beds

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Riverine systems can retain considerable amounts microplastics (MPs) through sedimentation, but their resuspension depends on multiple factors. These include particle characteristics (size, morphology, density) and hydrodynamic conditions (flow velocity, sediment bed structure). The resuspension of natural particles has been studied using various models for bed load transport, such as Shield theory, where the critical shear stress (function of flow velocity, viscosity, particle density and size) describes the hydrodynamic conditions at which a particle could be resuspended. However, little is known on if MPs behavior can also be predicted by these standard models due to significant differences in the properties

between natural particles and MPs. How specific river morphologies such as bed thickness and grain size impact resuspension is also unexplored, both in the context of 1) shielding/exposure effects exerted by grains on MPs deposited on the sediment surface and 2) MPs resuspension potential when buried within the sediment bed. Here we investigated MPs resuspension using a flume setup across various sediment grain sizes and bed thicknesses under different flow rates. The shielding effect was anticipated to be dependent by the grain size, with larger ones expected to have higher shielding potential than smaller ones. We found that larger grains limited MPs resuspension by changing the bed porosity instead. Large grains allowed MPs to deposit between the pores and cavities, limiting their resuspension. This effect was more pronounced with thicker bed where particles could deposit more in depth and be lost. Smaller grains lead to a more compact bed where MPs deposited on the surface were more exposed to the flow shear stress and more easily resuspended. When MPs were buried within the sediment bed, the main factor influencing their resuspension was the bed thickness, with thin layers allowing more MPs to resuspend than thicker ones. For both the scenarios tested, higher flow rates resuspended more particles than lower ones due to increased shear stress. Collectively, these results provide a better understanding of MPs transport in rivers by assessing the conditions which facilitate particle resuspension in various scenarios where bed load transport models do not directly apply. With the data collected we supported future modeling efforts to predict MPs fate, accumulation and transport in rivers.

1.10.P-Tu064 The Role of Flooding in the Distribution of Microplastics in an Arid Zone Stream

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Plastic waste has been detected on every continent and in every biome, from the deepest ocean trenches to deserts and mountains. Freshwater ecosystems are particularly at risk as sinks for microplastics (MPs). Factors that affect the distribution of microplastics in temperate areas are well studied, however, relatively little is known of processes in arid zones. The aim of this study was to investigate the effects human activities and flooding on the distribution of MPs in an arid zone stream, in the Sultanate of Oman. Sediment samples were collected from nine sites along the stream and two sites in the coastal floodplain. All sites had slow moving water, except Site 9, which was dry. Site 9 was in front of a flood retention dam designed to slow the flow of water to the sea. Samples were taken during the dry season and again shortly after flooding. Each site was categorized based on physico-chemical features and local human activities. Microplastics were separated from the sediments using a density gradient and were classified based on size, shape and colour. Polymers were identified using micro-Fourier Transform Infrared Spectroscopy (μ -FTIR). In both seasons, the most common shapes were fragments, followed by fibers, while most MPs were between 10-50 μ m. MPs abundance was positively correlated with total organic carbon, silt, and clay, and negatively correlated with sand content. Before flooding, most microplastics were associated with residential and agricultural zones, and lowest densities were in the middle reaches that were remote and hard to reach. The average concentration of MPs increased at most sites after the rain, suggesting that MPs in the surrounding watershed were washed into the stream. The most dramatic increase was at Site 9, the flood retention dam, where numbers increased from 927.7 ± 205.5 MP/Kg to 13166.7 ± 433.3 MP/Kg after rain, indicating that many particles accumulated at the dam base. As this site was dry, and predominantly consisted of fine clay, the MPs would eventually be distributed by wind, potentially creating a hazard to surrounding areas. Overall, we demonstrated that MP abundance is highly sensitive to local activities. The flood retention dam reduced the number of MPs reaching the sea, but also created a potential hazard by concentrating them in a dry basin where they will be distributed aurally. This study was funded by The Research Council Oman (RC/GRG-SCI/BIOL/23/01)

1.10.P-Tu065 Testing Single and Combined Environmental Stresses on their Potential to Induce Microplastic Fragmentation and Dissolution: Results on Abiotic Hydrolysis

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To optimally assess the fate of microplastics in the environment, it is essential to understand the mechanisms behind their degradation and fragmentation over time. Depending on the environmental compartment, microplastics encounter various environmental stressors (e.g., UV radiation, humidity, temperature, enzymatic activity) that can lead to polymer aging and subsequent fragmentation. Several decades of polymer aging research can serve as a basis to understand these complex environmental aging mechanisms. For microplastic fate assessment this knowledge needs to be expanded by investigating the fragmentation and release of secondary species, correlating them to the detectable changes in polymer properties. Abiotic hydrolytic reactions can be one potential environmental degradation pathway but the

impact of hydrolysis on polymer properties and fragmentation are rarely investigated. Determining the release rates of secondary micro- and nanoplastics, as well as dissolved organic carbon (DOC) under abiotic hydrolytic conditions, is essential for a better understanding of the issue. Here we conducted systematic comparative studies by testing the OECD TG111 and 3 more hydrolysis protocols on 7 types of microplastics (HIPS, LDPE, PP, PA-6, TPU, PLA, PET). We varied the temperature of artificial seawater (4, 22, 65 °C) and the pH (4, 7, 9) of ultrapure water. Various analytical methods were employed to quantify the time-dependent and size-selective release of DOC, as well as micro- and nanoplastic fragments up to 100 days and even 1 year aging. The chemical composition of the microplastic particles had the most significant effect on the type and quantity of species released, with only a few polymer types being affected by the treatments at room temperature. It was found that increased temperature had the most substantial influence on microplastic degradation and fragmentation. This study also revealed distinct polymer-derived DOC dynamics of PA-6 and PLA at 65°C. PA-6 exhibited stable DOC pool over 100 days, while PLA showed a dramatic increase. Liquid chromatography high resolution mass spectrometry (LC-HRMS) non-target analysis identified 561 unique features for PA-6, with minimal changes in chemical profiles, while PLA showed a dynamic chemical and kinetic evolution of degradation products.

The data obtained from these experiments are currently being utilized to enhance the parameterization of the mechanistic fragmentation model FRAGMENT-MNP.

1.10.P-Tu066 Microplastic Contamination at Various Early Developmental Stages of Marine Fish from Coastal Waters in Taiwan

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Microplastics (MPs) are widespread in coastal seas, posing risks to marine life. Planktonic fish larvae and juveniles, essential for fish population sustainability, are particularly susceptible to MPs, environmental stress, and predation. However, the effects of MP ingestion on larval fish remain poorly understood. This study investigated MP ingestion in 173 larval and juvenile fish from the coastal waters of Qieding, Taiwan. Gastrointestinal tracts were analyzed using alkali digestion, microscopy, and FT-IR analysis, revealing MPs in 21.39% of samples at an average of 0.35 ± 0.78 MPs individual⁻¹. A total of 59 MPs were identified across 20 fish species, with whipfin silver-biddy (*Gerres filamentosus*) showing the highest count (14 items; range: 0–3 MPs individual⁻¹). Polyethylene terephthalate (PET) was the most common polymer (44.07%), while fibers constituted the majority of particles (89.83%), primarily transparent (54.24%). Nearly half of all MPs measured between 1 and 5 mm. MPs were positively correlated with fish size ($R^2 = 0.038$, $p = 0.009$), and more advanced developmental stages, such as juveniles, showed a significantly higher incidence of MPs ($p < 0.001$). This trend indicates that MPs are more frequently ingested by juvenile or larger fish larvae. These findings provide essential insights into MP contamination in Taiwanese waters, highlighting potential impacts on the health of planktivorous larval fish and marine ecosystems. This study was financially supported by Grants from the National Science and Technology Council (NSTC 111-2611-M-291-001, NSTC 112-2611-M-291-003, and NSTC 113-2611-M-291-005) of the Republic of China to Ming-Yih Leu.

1.10.P-Tu067 Identification and Characterization of Microplastics in Mussels from the Bay of Biscay

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Recent studies classified the Bay of Biscay (BoB) as a marine litter accumulation zone, but knowledge about the characteristics of microplastics (MPs, 1 µm to 5 mm) in the region is scarce. Mounting evidence suggests that MPs are ingested by marine organisms, harming aquatic life and ecosystems. Furthermore, bivalves can be sources of MP exposure to humans since they are usually consumed as whole. While many studies focus solely on MPs, other anthropogenic particles (APs) such as cellulose, also exhibit persistence and potential hazards to marine environments. In this study, the presence and characteristics of APs including MPs were investigated in the whole flesh of marine mussels (*Mytilus* sp.) from the BoB, coming from the field and aquaculture. Field samples were collected in the Butroi-Plentzia estuary and in the Arriluze harbor (Basque Country-BC, Spain) in summer 2023 and in the Arcachon Bay, outside the

harbor (France) in spring 2023. Aquaculture mussels were obtained from Galicia (Spain) in autumn 2023 and in Mendexa (BC, Spain) in winter 2022. The whole tissue of 10 mussels per site was analyzed. Samples were digested with 10% KOH and sorted particles were characterized by visual analysis under a stereomicroscope followed by polymer identification by ATR-FTIR spectroscopy. APs were found in all individuals analyzed. APs mean concentration per individual ranged from 1.40 (± 1.20) to 7.60 (± 7.23) APs/ind, and APs mean concentration per fresh weight ranged from 0.20 (± 0.13) to 0.75 (± 0.60) APs/g fw. APs found were classified as fibers (97.9%) and fragments (2.08%). The length of fibers ranged from 342.40 (± 159.38) to 76.97 (± 390.67) μm and width ranged from 8.20 (± 3.46) to 19.46 (± 10.57) μm . The most prevalent colours detected were black and transparent. The most abundant polymer found was cellulose (60%). Six plastic polymers were detected: polyethylene terephthalate (PET), polyethylene (PE), polyamide (PA), polystyrene (PS), polyvinyl chloride (PVC) and polyisobutylene (PIB), of which PA fibers were the most common. In farmed mussels only PET and PA MPs were found, while a higher polymer diversity was observed in wild individuals. No significant differences were found in AP concentration and polymer composition among studied samples. These results can contribute to the definition of baseline levels of MP occurrence in mussels from the BoB. Work funded by the Spanish MICIU (project FIERA, ref PID2021-128600OB-I00, MCIN/AEI/ 10.13039/501100011033 and ERDF A way of making Europe), the Basque Government (grant to the consolidated research group IT1743-22 and a postdoctoral fellowship to NGS), the University of Bordeaux (internship grant to IG), and Euskampus Foundation (Laboratory for Transborder Cooperation LTC AquEus)

1.10.P-Tu068 The Role of Organismal Traits in Understanding Variability & Uncertainty in Reported Biota Microplastic Concentrations

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A significant body of ecotoxicological data has been developed for microplastics (MPs). However, there remain challenges in synthesizing and interpreting these data within the context of ecological risk assessment. While many of these challenges are being addressed, particularly those around study design, QA/QC, and reliability, one significant challenge remains - identification of relevant species for use in the development of ecological protection criteria (i.e., PNECs, HC5). Previous studies have attempted to derive these values, but without unanimity. This results in significantly different PNEC values as a function of the selected particle characteristics (size, shape, polymer), and uncertainty as to the relative sensitivities of different aquatic species to different MPs. This work summarizes the results of a systematic quality review of over 400 MPs biomonitoring studies using criteria previously developed by Hermesen et al., with minor modifications to integrate relevance and reliability more clearly for risk assessment & prioritization of potential future environmental monitoring programs. For studies that met the criteria, trait-based descriptors and particle characteristics and distributions were compiled for all species to provide guidance on species selection and environmentally relevant exposure profiles for informing future biomonitoring and risk assessment studies, highlighting knowledge gaps. The long-term objectives of this work are three-fold first, to systematically compile and evaluate species-specific traits (e.g. feeding guild, habitat, size, ingestion rate) data for a wide range of freshwater and marine species into a searchable database for use in identification of sentinel species for ecosystem health & quality monitoring, and ecological risk assessment. Second, this work is intended to provide a biologically-relevant framework against which the relevance of existing (and developing) MPs reference materials may be evaluated and additional studies prioritized. This can provide a systematic basis for the inclusion or exclusion of species or materials for the purpose of quantitative risk assessment in various environmental compartments. Third, available information on functional traits can inform the selection of relevant and efficient species for integrated biomonitoring programs in sea surface, subsurface, estuarine, and sediment environments.

1.10.P-Tu069 A New Active Device for Sampling Microplastics in Lakes: Development of a Floating Sampling Station

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The largest production of plastics lead to a significant increase of volume of plastic entering natural environment. More than 80% of the plastics entering into aquatic ecosystems derived from terrestrial ecosystem through rivers. Among freshwater ecosystems, lakes can be considered relatively closed ecosystems compared to other aquatic environments and therefore a potential long term reservoirs for microplastics (MPs). Recently there has been an increase in studies of freshwater water bodies as key

sources of drinking water and other paramount ecosystems services. Como basin in Lake Como (Northern Italy) is characterized by a relatively low water exchange, receiving water from two streams with wastewater treatment plants located along their course, which process domestic and industrial water, in part of textile origin. Within LIFE CASCADE project a number of activities are aimed at reducing the contribution of MPs and PFAS from the textile sector to surface water. For this reasons, in order to determine MP in water, it is important to be capable to sample large amount of water in specific geographic locations within the lake to study the fate of MPs. The aim of this study is to evaluate MP levels in Como town basin of Lake Como and the contribution of river output. In order to reach this goal a new MP sampling device was set up to collect larger volume samples at specific areas in the lake, rather than large transects obtained with a manta trawl. Several lake water samples were collected in Summer 2024 from the Como basin and from the main inflow rivers, Cosia and Breggia. Samples were analyzed with Py-GC-MS. Sediments were also collected. Overall, results were qualitatively in line with those found in literature although only few studies reported polymer concentrations ($\mu\text{g}/\text{m}^3$). The main polymers found were polyethylene (PE), polypropylene (PP), nylon (N66) and polyethylene terephthalate (PET). MP concentration was higher in littoral areas presumably affected by sediment re-suspension and the presence of largest particles than in open water areas. However, the low concentration of MPs in pelagic area could be also related to phytoplankton development and consequent suspended solid deposition which scavenges MPs from the epilimnion. River Cosia and Breggia concentrations and water flows allowed to estimate MP riverine contribution. Work is in progress and new monitoring campaigns are needed to evaluate MP fate and their temporal evolution in Lake Como.

1.10.P-Tu070 Qualitative and Quantitative Assessment of Microplastics Derived from Antifouling Paint in Effluent from Ship Hull Hydroblasting and Their Emission into the Marine Environment
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Microplastic (MP) pollution is a growing environmental concern, and ship paints have been identified as a significant source of MPs entering the marine environment. Hydroblasting, a common ship hull cleaning method, contributes to MP emissions, but its impact remains poorly understood. Understanding the characteristics and scale of MP emissions from hydroblasting operations is critical for assessing their contribution to global marine pollution. This study evaluated MPs generated during the hydroblasting of an ocean-going vessel, focusing on particle number, size distribution, polymer type, and mass. Additionally, an MP emission factor was calculated and extrapolated to estimate global emissions. Paint particles released from hydroblasting were analyzed for their physical and chemical characteristics. The emission factor (g/m^2) was derived by dividing the total emissions by the vessel's wetted surface area (WSA) (m^2). This factor was then used to estimate global MP emissions based on the WSA of ships worldwide and their cleaning frequency. Hydroblasting a vessel produced 4.3×10^{17} particles, with 99.9% smaller than 5 mm, predominantly comprising acrylic-based particles. Of the 44.1 kg of antifouling (AF) paint particles generated, 36.5 kg were smaller than 5 mm, including 18.2 kg of plastic emissions. The MP emission factor was determined to be $8.43 \text{ g}/\text{m}^2$. Using this factor, annual global MP emissions were preliminarily estimated at 665.6 tons, with 550.2 tons consisting of MPs. This study demonstrates the significant contribution of hydroblasting to marine MP pollution and emphasizes the urgent need for stricter regulations on hydroblasting operations and waste disposal practices to mitigate environmental impacts. These findings provide critical data for policymakers to develop effective measures to reduce MP pollution from ship maintenance activities, advancing efforts to protect marine ecosystems. This research was supported by the Korea Institute of Marine Science & Technology Promotion (KIMST), funded by the Ministry of Oceans and Fisheries, through the following projects: Land/sea-based input and fate of microplastics in the marine environment (RS-2022-KS221604) and Techniques development for management and evaluation of biofouling on ship hulls (RS-2021-KS211530).

1.10.P-Tu071 Integrating Metals in the Complex Array of (Micro)Plastic Additives: A Preliminary Screening in Conventional and Biodegradable Polymers

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Plastic pollution is a well-known environmental issue, yet the ecotoxicological implications, particularly concerning the chemical additives in plastics, are still underexplored. Attention has in fact increased for the characterization of chemical additives in plastics and their potential risks. The variety of additives (e.g., plasticizers, catalysts, fillers) is vast, and understanding their abundance is essential for developing effective safe designs of plastic materials. While significant research has been done on organic chemicals

and their leaching process, the data on inorganic additives (i.e., metallic and organometallic compounds) remain limited. In this study we analyzed the metal content in different types of plastics, focusing on their sources of enrichment and the associated risks. Specifically, we investigated pristine plastic pellets, recycled plastic pellets, and single-use plastic products made from both conventional and biodegradable polymers found in environmental compartments. Our goal is to better understand the presence of inorganic additives in these materials, their distribution across different polymer types, and their potential impacts. Our analysis revealed notable enrichment of metals particularly Ti, Al, Fe, and Zn across a range of plastic types, especially when comparing pre-production pellets to final consumer products. Polymers such as polyethylene (PE), polystyrene (PS), polybutylene adipate terephthalate (PBAT), polylactic acid (PLA), and polypropylene (PP) showed significant metal concentrations in the end-use products only, suggesting that these additives are incorporated during the final stages of production. PET samples also displayed elevated levels of antimony, while biodegradable plastics like PBAT and PLA exhibited specific trends related to tin and indium (the latter for PLA only): these elements resulted enriched already in the pellets, since they are used as catalysts in polymer production. This study provides the first comprehensive comparison of metal additives in both biodegradable and conventional plastics across different production phases. It highlights the need for caution when using plastic pellets for environmental risk assessments, as some may already contain additives not present in the final products. Additionally, the findings underscore the role of biodegradable plastics as potential carriers of metals in terrestrial and aquatic environments, raising concerns about their degradation and impact. This work was funded by the Research Council of Norway for the project PATTERN- PLAsTic effecTs on the Exchanges of nutRienTs and biological information in aquatic ecosystems (project number 345077).

1.10.P-Tu072 Physical and Chemical Stability of Recycled Plastic Aggregates as Natural Aggregate Replacement in a Cementitious Matrix

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The shortage of construction materials and the overflow of plastic waste have driven an increase in research aimed at substituting traditional aggregates with recycled plastic aggregates (rPA) in cementitious structures. However, the physicochemical stability of rPA may be impacted by the high alkalinity of cements, and thus pose questions on durability of such structures. The deterioration rate of two widely researched recycled plastics used as substitutes for aggregates, namely polyethylene terephthalate (rPET) and high-density polyethylene (rHDPE) was studied and the leachability of new emerging pollutants (NEP), such as per and polyfluoroalkyl substances (PFAS) and phthalates (PAE), from these rPA were measured in an aqueous cementitious matrix. Plastics were used in two particle size ranges: m=2.36 0.6 mm and μ =0.6-0.15 mm. Approximately 3 g of each polymer in the m-rPA and μ -rPA sizes were immersed in 30 mL of simulated concrete pore solution (SCPS) (13<pH) for 75 days. The deterioration rate of rPA during the alkali exposure was assessed through weight loss analysis, and the potential PAE leachability was examined using a gas chromatography mass spectrometer (GC-MS). The results revealed a significantly higher weight loss for μ -rPA with a higher deterioration rate in rPET than rHDPE. This suggested that ester groups in rPET underwent rapid cleavage due to alkaline hydrolysis and dissolved in SCPS. Moreover, a clear trend was observed in plastic degradation with prolonged alkali exposure; after 75 days, the weight loss of m-rPET, μ -rPET, m-rHDPE and μ -rHDPE were 9.8%, 39.0%, 1.1% and 7.9% respectively. GC-MS analysis revealed rHDPE contributed mostly to PAE release when exposed to SCPS, while μ -rPA released more PAE than m-rPA. Additionally, all rPA showed higher leachabilities of targeted PAE with increased time of exposure, most significantly being diethyl phthalate (leaching of up to 3.8 ± 1.1 ng mL⁻¹). Subsequent research will include leachability of short-, medium- and long-chain PFAS from rPA in alkaline-SCPS using liquid chromatography mass spectrometry. The findings offer important knowledge regarding the potential for polymer contamination upon degradation and NEP leachability from different plastic types under alkaline conditions. These results can be used to assess the environmental impact and sustainability of plastics when incorporated into a highly alkaline cementitious matrix.

1.10.P-Tu073 Conventional and Biodegradable Microplastics Influence Nutrient Cycling in the Aquatic Environment

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on nutrient uptake in soils, while relatively little attention has been paid to the aquatic environment. In this context, the aim of our study was to investigate the adsorption of phosphorus (in the form of orthophosphates) and nitrogen (in the form of ammonium, nitrite, and nitrate) on polyethylene (PE) and polybutylene adipate terephthalate (PBAT) microplastics prior and after biotic aging (i.e. with a developed biofilm). Biotic aging of microplastics was performed in freshwater from a local stream under controlled laboratory conditions. After 9 weeks, the percentage of biofilm was $33 \pm 3\%$ and $23 \pm 3\%$ for PE and PBAT microplastics, respectively. The maximum adsorption capacity of nutrients was reached after 24 h regardless of the type of microplastic and the results showed that the presence of biofilm significantly affected nutrient levels in the medium due to the adsorption by microplastics and utilization by microorganisms within the biofilm. For PE microplastics with biofilm, the adsorption capacity for ammonium nitrogen and nitrite was 10% and 13% lower, respectively, compared to pristine microplastics while no changes were observed for nitrate. For PBAT microplastics with biofilm, the adsorption of ammonium nitrogen was 20% lower than in untreated PBAT microplastics, while no changes were observed in the adsorption of nitrite or nitrate. The phosphorus concentration in the aquatic medium decreased by 18% and 13% when pristine PE and PBAT microplastics were present, respectively, indicating phosphorous removal by adsorption. On the other hand, the concentration of phosphorous decreased by 57% and 46% when PE and PBAT microplastics with biofilm were present, respectively, which was most likely due to adsorption and utilization by microorganisms. The results indicate complex interactions between microplastics and nutrients that vary with the presence of biofilm. Therefore, further studies are essential to elucidate the impact of these interactions on nutrient cycling in the aquatic environment, as they may also influence global biogeochemical cycles and greenhouse gas emissions.

1.10.P-Tu074 Effects of Plastics and Tire Rubber Leachates on Free-Living Freshwater Bacteria
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Plastic pollution poses a significant threat to aquatic ecosystems, e.g. as plastic leachates are contaminating water and posing toxicity risks for aquatic organisms. Plastic leachates, however, can also stimulate microbial growth, impacting carbon and nutrient cycles in aquatic ecosystems. Different synthetic polymer types have varying chemical make-ups, which result in variable leachate composition and thus effects on microorganisms. This study aims to assess i) how different synthetic polymer leachates affect free-living aquatic bacteria, and ii) how these effects vary at high and low nutrient conditions. To address these questions, leachates were extracted from five synthetic polymers, i.e. low-density polyethylene (LDPE), polypropylene (PP), polyvinyl chloride (PVC), starch-poly(lactic acid) (starch-PLA), and tire rubber by incubation in ultrapure water under UV radiation. Free-living ($<5.0 \mu\text{m}$) microbial communities of Lake Stechlin, Germany, were exposed to leachates, and the resulting communities were analyzed for changes in total microbial growth (TMG) using flow cytometry and for community shifts using 16S rRNA gene amplicon sequencing. To establish dose-response models, microbial communities from the lake were also exposed to a concentration series of PP, PVC, and tire rubber leachates, for which effects on TMG rates were observed. Synthetic polymers revealed differing effects on TMG and community composition depending on nutrient availability. For instance, at high nutrient concentrations, PP leachates didn't reveal any significant effects but led to significant community shifts and changes in TMG rates at low nutrient concentrations. In contrast, PVC leachates stimulated TMG of free-living communities at both nutrient conditions, though significant community shifts were only observed at low nutrient conditions. Starch-PLA leachates caused community shifts at both nutrient conditions despite having no effect on TMG, whilst tire rubber leachates showed inhibitory effects on TMG only at low nutrient conditions. These results highlight the importance of leachate quality and nutrient availability for our understanding of the effects of leachates on microbial growth and community composition. Our findings have important implications for understanding the impact of synthetic polymer pollution on microbial loop functioning and hence the biochemistry of various aquatic ecosystems, e.g. differing in trophic status. This work received funding from the European Union's Horizon 2020 Research and Innovation programme, under the Grant Agreement number 965367 (PlasticFate). The author(s) declare that there are no conflicts of interest regarding the publication of this paper. The research was conducted independently, without any influence from personal, financial, or organizational interests that could affect the study's outcomes.

1.10.P-Tu075 Identification of Metabolism and Degradation Patterns of 6PPD-quinone in Aerobic Soil Using Liquid Chromatography-Tandem Mass Spectrometry
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6PPD (N-1,3-dimethylbutyl-N-phenyl-p-phenylenediamine), a commonly used antioxidant in rubber, is primarily incorporated into tires to prevent oxidative degradation and enhance durability. However, it is released into the environment through tire and road wear particles. Upon exposure to sunlight, 6PPD undergoes photo-degradation to form its toxic metabolite, 6PPD-quinone, which has recently been identified as highly toxic to coho salmon, raising concerns about its environmental impact. In this study, we investigated the distribution and degradation mechanisms of 6PPD-quinone in aerobic soils. The research was conducted across three distinct soil types under controlled temperature conditions ($20 \pm 2^\circ\text{C}$) in the dark for 120 days. 6PPD-quinone was dissolved in acetonitrile and introduced into a flow-through system containing 80 g (dry weight) of soil. Throughout the experiment, each soil sample was collected in duplicate, extracted, purified, and concentrated before analysis by LC-MS/MS. The limit of detection (LOD) and limit of quantification (LOQ) for the target compounds were $0.02 \mu\text{g/kg}$ and $0.05 \mu\text{g/kg}$, respectively, with a calibration curve yielding a coefficient of determination (r^2) > 0.99 . Recovery was evaluated at three concentration levels (LOQ, $2 \times \text{LOQ}$, and $10 \times \text{LOQ}$), and the results indicated recoveries ranging from 97.7% to 113.9%, with relative standard deviations (RSD) below 20%. Soil samples were taken at multiple time points (0, 3, 7, 14, 30, 60, 90, and 120 days) for LC-MS/MS analysis after extraction, purification, and concentration. The average half-lives of 6PPD-quinone in the three soils were determined to be 43.0 to 94.9 days. Furthermore, a major metabolite was identified in each of the three soil types. The observed degradation pathways, degradation rates, and metabolites identified in this study contribute critical data for understanding the environmental behavior of 6PPD-quinone and its potential ecological risks in aerobic soils.

1.10.P-Tu076 Impact of UV Weathered Polystyrene and Polyethylene Microplastics on Microbially Mediated Nitrate Reduction in Floating Treatment Wetlands

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Floating treatment wetlands are a remediation option for waterbodies that receive nutrient pollution from agricultural and urban sources. Microplastics, a contaminant of emerging concern, are also prevalent in these sources and have the potential to alter microbially mediated nitrogen transformation, thus impacting the effectiveness of floating treatment wetlands. Previous experiments using virgin polyethylene and polystyrene microplastics to look at the influence of added microplastics on potential denitrification in floating wetland systems found a change in the potential denitrification rate dependent on microplastic size. This research indicates the potential for microplastic influence in floating wetland systems, but further exploration is needed to consider the impacts of altered surface characteristics due to environmental weathering of microplastics and the mechanisms causing the observed changes in potential denitrification. To create more environmentally relevant microplastics, the surface of polyethylene (200 μm) and polystyrene (200 μm and 30 μm) microspheres were weathered with simulated sunlight via xenon arc lamp over a spectral range of 300–800 nm at an irradiance of 650 W/m^2 and a 65°C BST for 30 days (720 hrs). Weathered microplastics were evaluated for changes in surface characteristics after the full weathering period. Rate of nitrate reduction was assessed for both weathered and virgin microplastics over two concentrations (1 mg/L and 100 mg/L). Microplastics were incorporated into microcosms with aggregated root samples collected from floating treatment wetland mats and monitored over five days to observe changes in nitrate concentration in the microcosm. Results from this research will help inform function and denitrification potential of floating treatment wetlands placed to provide remediation in contaminated waterbodies as well as contribute to potential trends on the impact of microplastic presence in environments that host similar denitrifying microbial communities such as natural wetlands and wastewater treatment plants.

1.10.P-Tu077 Unity Is Strength: Contrasting Responses in Group Formation Between the Green-algae *Tetradismus obliquus* and the Cyanobacteria *Dolichospermum flos-aquae* When Exposed to Nanoplastics

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Plastic pollution is a complex, global environmental problem, and its impact on freshwater ecosystems is not yet fully understood. Changes in freshwater phytoplankton community composition can be expected under nanoplastic pollution scenarios, due to the differential effects they pose upon the different phytoplankton groups. However, despite the efforts to synthesize the knowledge regarding nanoplastics effects on phytoplankton, a clear consensus has not been reached yet. This study shows contrasting effects on growth and morphology induced by nanoplastics (gold-doped polystyrene (PS) nanoplastics, $93 \pm 9 \text{ nm}$

in diameter) exposure on two different representatives of phytoplankton: the green-algae *Tetrademus obliquus* and the cyanobacteria *Dolichospermum flos-aquae*. Moreover, it explores how nanoplastics interact with these organisms at the cellular level, using transmission electron microscopy (TEM). We found that the species are affected differently: while the green algae was negatively affected by nanoplastics during its exponential growth phase, the cyanobacteria was negatively affected in the stationary phase and entered the decline phase earlier. Moreover, we observed that the proportion of cells in groups (coenobia or colonies) was different between treatments. When cyanobacteria were exposed to the highest concentration of nanoplastics in our experimental setup (45 mg PS/L), there was a higher proportion of cells in groups. Interestingly, this was the opposite for the green algae where the formation of groups followed a dose-response pattern and was lower in the presence of nanoplastics. When exploring possible mechanisms under TEM, we found that nanoplastics were attached to the cell wall of the green algae, which showed indications of damage. However, when analysing the cyanobacteria, although it was possible to observe nanoplastics in the close surroundings of the cells, we did not find nanoplastics directly attaching to them. Our study contributes to the understanding of the responses of phytoplankton species to nanoplastics exposure and the mechanisms behind those responses, to produce more accurate predictions of the impact of plastic pollution on freshwater phytoplankton communities. We acknowledge financial support by Kungliga Fysiografiska Sällskapet i Lund (Royal Physiographic Society).

1.10.P-Tu078 Evaluation of Photosynthetic Parameters of *Raphidocelis subcapitata* Exposed to Polystyrene Micro- and Nanoplastics

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Plastic pollution has been gaining attention in the last decades, especially regarding the effects of micro- and nanoplastics (MNPs) on biota and ecosystems. However, the impacts of MNPs on primary producers are less well understood, with the expectation that the lower the size of the plastic, the higher the risks of damage to organisms. In the present study, cells of the freshwater microalga *Raphidocelis subcapitata* (Chlorophyceae) in exponential growth phase were exposed to two concentrations of fluorescent polystyrene nanoplastics (50 nm NP 50 and 100 nm NP100) and microplastic (1 µm; MP): 0.1 (low) and 100 mg L⁻¹ (high). The photosynthetic activity of microalgae (maximum and effective quantum yields, photochemical and non-photochemical quenching, and rapid light curves) was assessed using a pulse amplitude modulated fluorometer (Phyto-PAM), and parameters were measured after 72 h exposure. Our results indicate that some parameters were not affected by the presence of microplastic, regardless of size and evaluated concentration. This was the case with the non-photochemical quenching NPQ and the parameter related to the activation of photoprotection mechanisms. Regarding the maximum quantum yield, both concentrations decreased the values in MP1, while only the high concentration affected the NP50 and NP100 treatments. The effective quantum yield was not affected by NP50 and MP1, and a lower value was observed at 100 mg L⁻¹ in NP 100. A slightly higher loss of heat and fluorescence by non-regulated non-photochemical energy loss was observed at the low concentration in MP1, with no difference at the high MP1 or in NP50 and NP100 treatments. The photochemical quenching was slightly affected at the high concentration in NP100 and MP1, with no differences at the low or NP50. Non-photochemical quenching was only affected at high concentrations in NP100. The rapid light curve parameters were not affected by NP50. On the other hand, high concentrations of NP100 decreased all the parameters (maximum electron transport rate rETR_{max}, light use efficiency, and saturation irradiance E_k), while high MP1 increased rETR_{max} and E_k. Even though some differences were observed, considering the different sizes and concentrations evaluated, a severe impact of MNPs in photosynthetic parameters was not observed, suggesting that photosynthetic machinery was not damaged by polystyrene MNPs. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 801370 and the Beatriu de Pinós postdoctoral programme funded by the Secretariat of Universities and Research (Government of Catalonia), project 2021BP00102 granted to the first author.

1.10.P-Tu079 Analysis of High Mass Polycyclic Aromatic Hydrocarbons (PAHs) Extracted from Microplastics Spilled in the Marine Environment

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In 2021, the container ship M/V X-Press Pearl caught fire and later sank off the coast of Sri Lanka. Over 1,600 tons of polyethylene pellets, also known as nurdles, were aboard the ship along with 25 tons of nitric acid, which began to leak and is suspected to be the cause of the fire that led to the sinking. To exacerbate the problem this fire included fertilizers and explosives being transported as well as the flame retardants deployed. When combined with abundant, naturally occurring compounds found in the marine environment, the need arises to characterize the composition of intact, burned, and broken pieces of the spilled plastic later collected from kilometers of Sri Lankan beaches. Samples of relatively intact, burnt, and aggregated combustion remnants of microplastics were extracted using a microextraction method involving sonication in DCM and the addition of multiple surrogates. Extracts were analyzed on a Xevo TQ Absolute (Waters Corporation) tandem quadrupole system using N₂ CID gas and fitted with the Atmospheric Pressure Gas Chromatography (APGC) source in positive ion mode using charge exchange ionization and dry N₂ reagent gas. The focus of this work was on high mass PAHs (314 - 424 Da, 6 - 11 rings). However, an overlapping range of PAHs (228 - 302 Da, 4 - 6 rings) was included to facilitate comparison across techniques and with other published results. NIST Coal Tar SRM 1597 was used to perform method development as it was anticipated to include representative analytes. The final method using a high temperature (400°C) stable column resulted in all analytes being eluted in <23 minutes while still providing satisfactory chromatographic resolution for the majority of high mass analytes. Development of MRM transitions was based on extracting prospective high mass PAH molecular weights from multiple sources and using NIST 1597 to optimize response for the most prominent peaks in each RT range. Subsequent quantification of the overlap region analytes found in 1597 were within 10.2% of the reference values for 8 of 9 analytes, with coronene being an outlier (+58.9%) due to coelution. Combustion remnant and burnt samples contained >500x and >250x, respectively, of the levels of high mass PAHs found in white nurdles. Quantification of high mass PAHs shows that white samples have <1%, burnt have 18.2%, and CR has 40.7% by mass when comparing the cumulative response for all analytes detected in 1597.

1.10.P-Tu080 Investigating the Complex Impacts of Plastic Pollution in Aquatic Ecosystems: Insights from a Laboratory Experiment

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The presence of plastic litter in freshwaters has raised concern about its long-term implications for water ecosystem functioning. However, while increasing knowledge is collected regarding ecotoxicological implications, wider direct and indirect environmental consequences of plastic pollution are still poorly understood. In this study, we investigated the role of plastic as a new, anthropogenic substrate for microorganisms affecting the microalgal community composition and functioning in a lab-based experiment. We crafted plastic fragments of different polymer types and incubated them with water collected in an urban pond, in order to have them colonised by a microbial community. Then, we transferred these fragments into experimental batches containing environmental samples of a microbial community from a pristine lake to simulate the potential longitudinal transport of plastic in unpolluted water bodies. Plastic fragments were added at 2 different concentration levels in the experimental batches. The experiment lasted 21 days in total, and we sampled water and plastic weekly to assess major and minor trace metals, the growth of photosynthetic microorganisms, extracellular polysaccharides and the microbial community composition. We observed changes in the algal community composition and marked chemical alterations driven by the presence of plastics. This is due to the dispersal of algal species from the biofilm and the specific colonisation of plastic fragments by some of the organisms in the natural pelagic community, favouring specific traits. We observed a lower abundance of microalgae in the pelagic phase, a faster depletion of nitrates and a significant change in the extracellular polymeric substances available in the dissolved phase at increasing plastic concentrations. We also observed the leaching of some trace metals (e.g., lead) from plastic. We also observed endpoints that were not affected by the concentration of plastic, such as some minor nutrients (e.g., sodium, magnesium and sulphates) and the enzymatic activity of the microbial community. This experiment highlights the complex array of mechanisms by which plastic can alter the ecosystem functioning and warns the community about the broader environmental risks that should be taken into account when considering plastic pollution in aquatic ecosystems. This work was funded by the Research Council of Norway for the project PATTERN-PLAsTic effectTs on the Exchanges of nutRienTs and biological information in aquatic ecosystems (project number 345077).

1.10.P-Tu081 Ecotoxicological Assessment (*Daphnia magna*) of Safe, Sustainable and Recyclable by Design SURPASS Polymeric Alternatives for Food, Building, and Transport Applications

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Plastics have become indispensable across many sectors due to their durability and versatility. However, their persistence in the environment poses severe health and ecological risks, as they degrade over centuries, fragmenting into micro- and nano-plastics (MNPs), and leaching toxic additives that pollute ecosystems and potentially disrupt human health. Currently, 70% of plastic waste in Europe is landfilled or incinerated, underscoring the need for safer and more recyclable alternatives. The SURPASS project addresses this challenge by developing Safe, Sustainable, and Recyclable by Design (SSRbD) polymeric materials. Following the European SSbD framework, the new developed materials focus in three high-demand sectors: a) building (replacing PVC in windows frames by bio-sourced polyurethane resins); b) transport (using lightweight epoxy-vitrimers to replace train metal components); and c) packaging (replacing non-recyclable films by recyclable multinano layered films). These alternatives are intended to reduce environmental impact while meeting industry performance standards. To assess the environmental safety of these new developed SSRbD materials, *Daphnia magna* was selected as model organism for acute aquatic toxicity, measuring EC50 immobilization values (following OECD202 guideline). 3 hardeners, 5 different combinations of resins + flame retardant, and leachates extracted from 4 different final composites were tested in the transport sector. In the case of building sector, 5 different bio-based polyols and 3 catalysts were studied, along with other 5 non-replaceable components. In the case of packaging sector, toxicity of the leachates of 4 multilayered films were tested. Safer materials were selected (regarding acute aquatic toxicity) for each of the component s types. The obtained values shall be integrated with other hazard endpoints as cytotoxicity, oxidative stress, epigenetic modifications, genotoxicity, and endocrine disruption to generate a final hazard score of each material, allowing the selection of the materials that offer more comprehensive environmental and human safety. This study is crucial in driving safer and greener innovations in the plastics industry. By rigorously evaluating and advancing less hazardous polymers, the project sets a benchmark for sustainable design, fostering broader adoption of SSRbD principles and supporting a circular economy transition. This project has received the funding from the European Union s Horizon Europe research and innovation program under grant no. 101057901 Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

1.10.P-Tu082 Combined Toxicity of Antibiotic Ciprofloxacin and Polylactic Acid Microplastics to *Daphnia magna*: Role of Dissolved Humic Acid

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Biodegradable microplastics (BMPs) can exhibit toxic effects on aquatic organisms, similar to conventional plastics, through pollutant adsorption and ingestion. This study investigates the adsorption of humic acid (HA) and ciprofloxacin (CIP) onto BMPs (polylactic acid, PLA) and their combined toxicity to *Daphnia magna* during a 17-day chronic exposure. CIP, a fluoroquinolone antibiotic, is known to induce mitochondrial genotoxicity in *Daphnia magna*, affecting growth and reproduction. We hypothesized that antibiotics adsorbed onto BMPs would amplify toxicity in the gut of *Daphnia magna* and that HA-BMP interactions would increase CIP adsorption sites, further enhancing toxicity. The results provide insights into the interactions between aquatic organic matter, BMPs, and antibiotics, highlighting the combined toxic mechanisms and implications for environmental risk assessment. This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (NRF-RS202400335682).

1.10.P-Tu083 Ecotoxicity of Microplastics from Biodegradable Mulch Films: Metabolomic Analysis on *Daphnia magna*

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Biodegradable mulch films have been introduced in agriculture as a sustainable alternative to conventional plastic mulches to reduce waste and improve soil health. However, concerns have emerged regarding the environmental impact of these materials on nearby freshwater ecosystems. As these films degrade, they may release various organic compounds and microplastics, potentially becoming bioavailable to aquatic organisms such as *Daphnia magna*, leading to unforeseen ecological interactions. Metabolomics has proven to be one of the most powerful tools for identifying mechanisms of toxicity. Therefore, the

application of metabolomics is expected to reveal the mechanisms of the toxic effects of biodegradable microplastics (BMPs) derived from biodegradable mulch films on metabolite expression in *D. magna*. This study investigated the chronic toxicity of BMPs considering relevant scenarios (5 mg/L, <60 μ m, using pristine and original particles), using the water flea *D. magna* as model organisms, respectively, due to their ecological relevance in freshwater environments. Adults (5 d old) daphnids were exposed for 21 days to identify the impact of BMPs on metabolites of *D. magna*. Growth (mother's body length), consumption (ingestion rate, energy reservation) parameters, and metabolomics were measured to evaluate the effects of BMPs and additives. This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (NRF-RS202400335682).

1.10.P-Tu084 Effect of Conventional and Biodegradable Microplastics on *Daphnia magna* Population

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Biodegradable plastics (BPs) have been raising concerns lately regarding the generation of biodegradable microplastics (MPs) from incomplete biodegradation in the natural environment. Thus, evaluating the ecological effect of biodegradable MPs in aquatic ecosystems is essential for understanding the environmental effect of BPs. The present study investigates the population-scale effect of conventional (polyethylene terephthalate, PET) and biodegradable (polyhydroxybutyrate, PHB) MPs to *Daphnia magna* population. PET MPs significantly lowered population biomass compared to control, while PHB MPs did not. Gut microbial community analysis showed that PHB MP changed community structure compared to control and PET. This implies that biodegradable MPs may have a different mode of action than conventional MPs by altering the gut microbial community of zooplanktons, possibly acting as an additional carbon source or as an additional symbiotic relationship. This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (NRF-RS202400335682).

1.10.P-Tu085 Role of Humic Acid in the Combined Toxicity of Biodegradable Polylactic Acid Microplastics and the Pesticide Diflubenzuron to *Daphnia magna*

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The increasing need to address plastic pollution has led to the growing production and use of biodegradable polymer-based plastics. Although biodegradable plastics are known to completely decompose into water, carbon dioxide, and biomass under specific conditions, they may persist in the environment as microplastics under various environmental conditions, similar to conventional plastics. Recent studies have shown that biodegradable microplastics may exert comparable or even greater ecological toxicity effects on animals and plants than non-biodegradable microplastics. They can also act as carriers for environmental pollutants in a similar manner. Organic matter can interact with microplastics and pollutants in aquatic environments, influencing their adsorption behavior. Several studies have demonstrated that the interactions between organic matter, microplastics, and various pollutants can either mitigate or exacerbate toxicity to aquatic organisms. However, the role of organic matter in relation to biodegradable microplastics and pollutants remains insufficiently explored. Diflubenzuron (DFB) is an insecticide with a toxic mechanism that interferes with exoskeleton formation in insects. It has been reported to cause mortality, reproduction inhibition, and growth impairment in the aquatic crustacean *Daphnia magna*. In this study, we evaluated the role of humic acid, a representative organic matter, in the combined toxicity of the biodegradable plastic polylactic acid (PLA) and DFB. Adsorption test were conducted to examine the interactions between biodegradable microplastics and diflubenzuron in the presence of humic acid. Additionally, acute toxicity test performed using *D. magna*. After 48 hours of exposure, we recorded toxicity results (immobilization and mortality) and collected organisms to analyze oxidative stress, and chitin content related to the toxic mechanism of DFB. The findings of this study provide valuable insights into the interactions between organic matter and biodegradable microplastics and their ecotoxicological risks. Furthermore, they contribute to a better understanding of the role of biodegradable microplastics as vectors for pollutant transport in the environment. This research was supported through the National Research Foundation of Korea (NRF) funded by Basic Science Research Program from Ministry of Science and ICT of Korean government (NRF-2022R1C1C2009130).

1.10.P-Tu086 Egestion Rates of Microplastics in *Daphnia magna* Depending on Food Uptake

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Micro-sized plastics have been concerned about potential effects on aquatic organisms. Microplastics can be ingested by aquatic organisms, both filter feeders and non-filter feeders. Waterflea is one of the filter

feeders, and previous studies have shown waterflea can ingest various types of microplastics. Most research have been reported ingestion and egestion of microplastics on waterflea using microscopy images. Since gut fullness images cannot be quantified the amount of microplastics, this study aimed to quantify the ingestion and egestion amounts of microplastics using fluorescence microplastics. Egestion rates of microplastics were observed under presence and absence of food conditions. *Daphnia magna* was exposed to 1 mg/L of 1.5 μ m sized green fluorescent polymer microspheres for 24 hours then, they were transferred to clean media for 24 hours under presence or absence of food conditions. Egested microplastics were detected using fluorescent intensity. As a result, microplastics were egested from *D. magna* faster under the presence of food than the absence of food. *D. magna* can be egested the microplastics under food supplied conditions. This method can be easily quantifying the amount of ingested and egested microplastics than using the gut fullness of image. Food co-exposure system also need to consider the microplastic effects on aquatic organisms. This research was supported by Korea institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (KIMST-20220383).

1.10.P-Tu087 The Long-Term Effects of Plastics in Semi-Natural Population of Zooplankton

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Multiple studies on the ingestion and effects of primary and secondary plastic fragments on zooplankton have been performed in laboratory conditions. However, the knowledge on the effects of naturally fragmenting microplastics (MP) in situ is still scarce and impacts over generations are rarely assessed. In this experiment effects of plastics on the fitness of cladoceran communities, based on the production of resting eggs, were studied in a mesocosm study spanning over two summer periods. The experiment was carried out with local, field-collected cladocerans (*Daphnia magna*) on an outer archipelago island (Furuskär, SW coast of Finland) with multi-generational exposure of macro- and microplastics. Altogether 42 experimental units (glass containers) were prepared: 14 control units with only cladocerans (40 ind. *D. magna*), 14 units with *D. magna* and weathered MP originating from a polyethylene (PE) tarp and 14 units with *D. magna* and a piece of the same tarp. At the end of the first summer adult cladocerans and the resting eggs were collected and counted. The resting eggs were stored in glass vials over winter in natural conditions. The experiment was repeated in the following summer with a similar set up, with the exception that instead of using field-collected cladocerans, the ephippia from the previous year were used to initiate the cladoceran communities in the units. At the end of the experiment *D. magna* and ephippia were counted, water and sedimented organic matter samples collected for the additives (UV-stabilizers) and microplastic analyses. Ephippia were kept in cold (+5 °C) and dark over winter, and a hatching rate experiment carried out in the following spring. Preliminary results indicate that both MP and leachates from larger plastics have a negative impact on the resting egg production. These findings increase knowledge on the role of different exposure pathways of plastic litter in aquatic invertebrate communities' generations.

1.10.P-Tu088 Ingestion and Depuration Rates of Metal-Doped Nanoplastics (Pd-PS NPs) in Pelagic and Benthic Copepods: Implications for Trophic Transfer

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The fragmentation of environmental plastics into nanoplastics (NPs, <1000 nm) and shedding of nanoplastics from plastic surfaces has raised concerns regarding their distribution and impacts in marine ecosystems. Many studies have reported NPs ingestion and effects on marine invertebrates, often utilizing fluorescently labeled NPs. However, the reliability of these methods has been questioned due to the potential leaching of fluorescent dyes into tissues. Moreover, most research has been conducted individually in a few model species like zebrafish, sea urchins, or mussels, which do not reflect the diversity of the species in marine communities. Copepods, the most abundant marine animal group, play a key role in energy transfer within food webs. However, to the best of our knowledge, no studies have investigated NPs accumulation across different copepod species. This study aimed to quantify NPs ingestion and depuration in various pelagic and benthic copepods with distinct feeding behaviors: ambush feeding, cruising, current feeding, and benthic grazing. For this, we exposed the copepods *Temora longicornis*, *Centropages hamatus*, *Apocyclops royi*, and *Tisbe battagliai*, that represent the major copepod groups (calanoids, cyclopoids, harpacticoids) to 150 nm metal-doped polystyrene NPs (Pd-PS NPs). Following foodborne exposure, copepods were transferred to clean seawater for depuration. NPs content was quantified at multiple time points during the exposure and depuration period using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Our preliminary results show species specific rates for

NPs accumulation and depuration. We hypothesize that species such as *A. royi* (ambush feeders) and *T. battagliai* (benthic grazers) will retain NPs longer due to their feeding behaviors and slower gut transit times, compared to calanoids like *T. longicornis* and *C. hamatus*. These findings highlight the importance of species-specific traits in NPs accumulation and emphasize the need to consider biological and physiological factors when designing experiments of trophic transfer of NPs.

1.10.P-Tu089 Distinct Chronic Toxic Effects of Fragmented and Fibrous Polyethylene Terephthalate Microplastics on Marine Amphipod *Monocorophium uenoi*

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Microplastic (MP; 1 5000 µm) pollution has emerged as a critical environmental concern, with fragments and fibers being the most abundant shapes of MPs in marine ecosystems. Despite their prevalence, toxicological studies on benthic organisms remain poorly understood. Therefore, this study aimed to distinguish the chronic toxicity of fragmented and fibrous polyethylene terephthalate (PET) MP to *Monocorophium uenoi*. Chronic toxicity tests (n = 3) were conducted with 34 *M. uenoi* (size: 300-500 µm) in 1 L glass beakers containing 200 g of sediments and 800 mL of artificial seawater (salinity 30 psu). *M. uenoi* were exposed to fragmented (small, medium, and large size) and fibrous (short and long length) MPs for 35 days. At the end of the experiment, mortality, MPs accumulation, behavioral activities (distance, velocity, energy distribution (carbohydrate, protein, lipid), and oxidative stress (lipid peroxidation (LPO) and total antioxidant capacity (TAC)) were evaluated. All concentrations (0.0005 and 10 mg L⁻¹) of Fragment M induced significant mortality with MP accumulation at 10 mg L⁻¹, while no accumulation of fibrous MPs was observed in *M. uenoi*. For behavioral activities, all concentrations of Fiber S showed significant decreases compared to the control, while fragmented MPs decreased behavior only in dark conditions or at high concentrations (10 mg L⁻¹). Regarding energy distribution, fragmented MPs induced significantly greater changes than fibrous MPs, with energy being utilized for immediate energy source, growth, and immunity. Furthermore, fragmented MPs significantly increased both LPO and TAC, indicating activation of antioxidant defense mechanisms in response to fragmented MP-induced oxidative stress. In contrast, fibrous MPs only induced significantly low TAC without changes in LPO, suggesting different stress mechanism. These findings highlight the distinct toxic effects and mechanisms between fragmented and fibrous PET MPs in marine organisms. This understanding provides crucial insights for environmental risk assessment of differently shaped microplastics and supports the development of more targeted management strategies for marine plastic pollution. This work was supported by Risk assessment to prepare standards for protecting marine ecosystem of Korea Institute of Marine Science & Technology Promotion (KIMST), funded by the Ministry of Oceans and Fisheries (KIMST-20220383).

1.10.P-Tu090 Biological Response and Molting Regulation of Whiteleg Shrimp to Microplastic Fibers

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With the continuous release of plastic particles, the marine ecosystems are under threat from the microplastic contamination and these microplastics eventually reach to the ecological receptors consumed as food sources. *Penaeus vannamei* (whiteleg shrimp), one of the valuable food sources in the ocean, were exposed to polyethylene terephthalate based microplastic fibers (PET MFs) for 21 d, and the biological responses were observed. We revealed that PET MFs induced growth inhibition and altered molting cycle with the changes of exoskeletal chromatophores and hepatopancreas. By analysing the transcriptomes and metabolomes, the alterations of biosynthesis in chitin and molting hormone related molecules affected to the molting cycle and exoskeletal impacts. These results improve our understanding of biological responses and metabolic regulation of *P. vannamei* to PET MFs, ultimately suggesting the need to protect ecological receptors from the microplastic contamination for the environmental sustainability and economic value as food sources. This research was supported by Risk assessment to prepare standards for protecting marine ecosystem of Korea Institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (KIMST-20220383).

1.10.P-Tu091 Comparative Toxicity of Dialyzed Nanoplastic and Leachates in Zebrafish Larvae

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Amid growing concerns over the ecological and health impacts of plastic pollution, nanoplastics have emerged as a significant environmental hazard. A key debate centers on whether the observed toxicity is due to the plastic particles themselves or their leachates. In this study, we address this issue by

comparatively analyzing the toxic effects of small-sized (15 nm) dialyzed nanoplastics and their leachates on the development of zebrafish (*Danio rerio*) larvae up to five days post-fertilization. We also investigated the impacts of plain polystyrene particles versus those with an anionic carboxyl (COOH) surface charge. Extensive morphometric analyses of sixteen endpoints revealed that both nanoplastics and their leachates adversely affect organ development in zebrafish larvae. Notably, dialyzed polystyrene (DPS) and carboxylated polystyrene (DPSCOOH) induced significantly higher mortality rates compared to their leachate counterparts. DPS had a more pronounced effect on body curvature and length than its leachate. Furthermore, significant differential impacts between nanoparticles and their leachates were observed in neurodevelopment (eye, head, otolith, and neuronal areas), digestive system development (stomach area, intestine length, and yolk area), and muscular development (muscle area). Behavioral abnormalities in larvae from both the dialyzed and leachate exposure groups accompanied these morphological defects. Future chemical characterization of leachate constituents will aid in identifying specific chemicals responsible for the observed toxic effects. This study was funded by the Sapere Aude Research Leader program from the Danish Council for Independent Research (No.0165-00056B).

1.10.P-Tu092 Effects of Field-Collected Microplastic Particles on Zebrafish (*Danio rerio*) Embryos: Importance of Particle Size

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Due to the global surge in plastic production, the evaluation of the environmental impact of plastic waste has emerged as a central focus in ecotoxicology. Notably, smaller plastic fragments, termed microplastics (MPs), have been identified across diverse aquatic ecosystems. Due to their small size and low biodegradability, MPs can easily be ingested by organisms and transferred along food webs. Ingestion may not only bear physical risks, but could also implicate chemical hazards, as MPs can convey man-made environmental contaminants, including pharmaceuticals and pesticides. Consequently, MP ingestion has frequently been linked to adverse effects on mortality, growth, feeding behavior, endocrine functions, and immune responses. Given that many mechanisms driving MP toxicity remain poorly understood, additional research is essential to evaluate the risks MPs pose to aquatic life. To study the biological effects of organic extracts from MPs, wild-type zebrafish (*Danio rerio*) embryos were used as a non-protected vertebrate model. The MP samples, collected as part of the JPI Oceans project RESPONSE (<https://www.response-jpioceans.eu/>), consisted of three different size fractions: 20 - 50 µm, 100 - 200 µm, and 500 - 1000 µm. These samples were sourced from European coastal waters including the Tyrrhenian and Adriatic Seas as well as the Bay of Biscay. Organic contaminants adsorbed to MPs were extracted using n-hexane. Toxicological endpoints included (1) acute toxicity; (2) induction of ethoxyresorufin-O-deethylase (EROD) activity through the aryl hydrocarbon receptor-mediated activation of cytochrome P450-dependent monooxygenases (CYP1A); (3) acetylcholinesterase activity; and (4) changes in the optokinetic response, with a focus on differences in effects related to particle size. Effects proved particle size-related and include symptoms of acute toxicity, a reduction of body length and induction of EROD activity, as well as changes in swimming activity. Together, these experiments provide insights into the toxic mechanisms and effects induced by MP extracts in zebrafish embryos, potentially enhancing the environmental hazard and risk assessment of MPs. This study has received financial support within the JPI-Oceans-associated project RESPONSE from the German Ministry for Education and Science (BMBF) under contract no. 03F0853A.

1.10.P-Tu093 Nanoplastic Alleviates Neurotoxicity of Mercury in Zebrafish through Regulating the Metabolic Pathways of Brain-Gut-Microbiota Axis

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Mercury is a high neurotoxic environmental pollutant that affects human health. However, the impact of emerging environmental pollutants such as micro-and nano-plastics on the neurotoxicity of mercury remains unclear. In this study, the 'brain-gut-microorganism' axis of zebrafish was used as the object to explore how nanoplastics affect the neurotoxicity of mercury on zebrafish by integrating metabolomics and microbiomes. The results showed that single mercury exposure significantly increased the anxiety behavior of zebrafish and slowed zebrafish swimming capacity, but the co-exposure of nanoplastics alleviated the anxiety behavior and exercise injury of zebrafish caused by mercury exposure. Moreover, the co-exposure of nanoplastics and mercury also significantly reduced the accumulation of mercury in the brain and intestine of zebrafish. Microbial and metabolomics analysis showed that nanoplastics could not only alleviate the disorder of intestinal microflora in zebrafish caused by mercury exposure, but also

significantly reduce the intestine microbial diversity. Compare with single mercury exposure, the co-exposure of nanoplastics can also improve the brain metabolic disorders and slow down the neurotoxicity of mercury by regulating glycerophospholipid metabolism, arachidonic acid metabolism and γ -linolenic acid metabolism in the 'brain-gut-microorganism' axis. In summary, nanoplastics alleviate the neurotoxicity of mercury exposure to zebrafish by regulating the key metabolic pathways of the 'brain-gut-microbiota' axis. Our results provide a new understanding of how nanoplastics interfere with the neurotoxicity of mercury in zebrafish. The work was supported by the National Natural Science Foundation of China (No.22066026), the Applied Basic Research Foundation of Yunnan Province (No.202401AS070144).

1.10.P-Tu094 Polyvinyl Chloride Microplastics Modulate Fipronil Accumulation and Toxicity in Zebrafish

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Microplastics (MPs) can transport and facilitate the transformation of co-existing contaminants, altering their bioaccumulation and toxic effects in organisms. This study examined the adsorption and transformation of fipronil in the presence of polyvinyl chloride (PVC) MPs (150–530 μ m) and assessed their individual and combined effects on zebrafish. PVC MPs were found to adsorb fipronil with a maximum capacity of 7393 ng g⁻¹ and facilitated its transformation into fipronil-sulfone and fipronil-desulfinyl. Zebrafish exposed to dietary fipronil alone (70 ng g⁻¹) for 14 days accumulated fipronil and fipronil-sulfone at total levels of 0.17 and 0.30 ng g⁻¹, respectively. In contrast, combined exposure with MPs (1% w/w) increased the bioaccumulation of fipronil and fipronil-sulfone to 0.53 and 3.25 ng g⁻¹, respectively, and additionally led to the accumulation of fipronil-desulfinyl at 0.77 ng g⁻¹. Both single and combined exposures enhanced zebrafish swimming behavior, with synergistic effects observed initially (days 1 and 3). The impact on antioxidant enzyme activities (CAT and SOD) was more pronounced in the groups exposed to MPs and the combined treatments, highlighting the dominant role of MPs in inducing oxidative stress. Principal coordinate analysis of behavioral and enzyme activity outcomes revealed distinct separations among the control, single MPs, single fipronil, and combined exposure groups. Overall, our findings demonstrated that PVC MPs significantly enhanced bioavailability and altered the metabolic profile of fipronil in aquatic organisms, leading to notable physiological and biochemical changes.

1.10.P-Tu095 Multi-omics Insights into the Combined Ecotoxicity of Antibiotics and Microplastics in Zebrafish under Acute and Chronic Exposure

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Emerging contaminants pose growing environmental concerns due to their inadequate regulation and unclear ecotoxicity. Among these emerging contaminants, microplastics and antibiotics have obtained increasing scientific attention. Given the ubiquity and sorption ability of microplastics, investigating the ecotoxicity of their co-occurrence is both realistic and crucial. However, our current understanding on their combined ecotoxicity remains limited. This study aims to perform comprehensive ecotoxicity assessment on antibiotics, microplastics and their co-occurrence, by multi-omics approach, using adult zebrafish (*Danio rerio*) as a model organism under both acute and chronic exposure. In this study, Oxytetracycline (OTC) and Polyethylene (PE) fragments (<500 μ m) were selected as representative antibiotics and microplastics, due to their wide application and environmental prevalence. Adult zebrafish was exposed to OTC alone, PE alone, or their combination for 4 days (acute exposure) or 30 days (chronic exposure). Subsequently, DNA, RNA, and metabolites were extracted accordingly and processed by sequencing or Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) to retrieve gut microbiome, transcriptomics, and metabolomics data. OTC and PE microplastics exhibited a significant synergistic effect on the transcriptomic profile of adult zebrafish under acute exposure, highlighting combined toxicity related to cell death, genotoxicity, and reproductive toxicity. This synergism was less evident in the gut microbiome and metabolomic profiles. However, all three omics layers consistently revealed the distinct ecotoxicity of co-occurring OTC and PE. Greater within-group variation was observed in gut microbiome of co-occurrence group, indicating potential gut microbiome dysbiosis. Metabolomics indicated distinct combined impacts, including dysregulated arachidonic acid and carbohydrate metabolism. The observed synergistic effect raises the environmental concerns of co-occurring antibiotics and microplastics, but was limited to acute exposure. Similarly, toxicological impacts under chronic exposure were generally lower, indicating the potential adaptation. However, chronic OTC exposure induced additional toxicity on reproduction and cell development, which was not identified

under acute exposure. Further research is recommended to explore the mechanisms underlying the synergistic effect and biological response to external exposure.

1.10.P-Tu096 Application of Probabilistic Species Sensitivity Distribution Modelling to Characterize Microplastic Risk for Marine and Freshwater Environments

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Reliable accounting for the diversity of microplastics (MPs) in hazard assessments involves the use of ecologically relevant metric (ERM) alignments requiring distribution data of physical particle attributes and organism bio-accessibilities. While eco-toxicological thresholds have been developed using this approach, uncertainties associated with the alignments have not been fully quantified. The present study utilized a probabilistic species sensitivity distribution (pSSD) model based on the updated Toxicity of Microplastics Explorer (ToMEx 2.0) database to derive hazard thresholds for MPs, while accounting for uncertainties due to alignments. Aligned No Observed Effect -concentrations (NOECs) were used to populate pSSDs to derive thresholds in a four-tier management framework based on the food dilution and tissue translocation-mediated effects ERMs. pSSDs differ from traditional SSDs by avoiding the use of predefined distributions to model species sensitivities, instead utilizing empirical calculations with the complete data set therefore more accurately representing underlying data. The ToMEx 2.0 dataset comprises 12,878 data points from 289 studies covering species across marine (n = 101) and freshwater (n = 67) environments. The dataset was filtered using pre-defined quality criteria, resulting in a total of 398 data points from 58 studies, with 25 marine and 19 freshwater species. Thresholds for marine and freshwater environments were derived separately using previously defined four-tier-management criteria. The resulting ERM-aligned hazard thresholds ranged from 1×10^{-4} to 4 particles/L, and 1 to 4,000 particles/L for marine environments, and 8 to 1,000 particles/L, and 500 to 20,000 particles/L for freshwater environments for food dilution and tissue translocation-mediated effects, respectively. Threshold uncertainties based on alignment of Monte Carlo simulations were lower for freshwater than marine environments, with percent relative standard deviations ranging from 24% to 270% for marine thresholds, and 21% to 130% for freshwater. This study is the first application of fully probabilistic methods to derive ERM-based MP hazard thresholds. We further explore the variability, uncertainty, and limitations of these thresholds through quantitative global and local sensitivity analyses, as well as qualitative discussions regarding study quality and data availability.

1.10.P-Tu097 Horizontal Gene Transfer Antibiotic Resistance Gene in Presence of Bio-Plastics: PLA and PHBV

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It is well known that microplastics (MPs) from conventional plastics facilitate horizontal gene transfer (HGT) of antibiotic resistance genes (ARG). This study investigates whether MPs derived from bio-based plastics, such as polylactic acid (PLA) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), exhibit similar behaviour. We used a trimethoprim-resistant *Escherichia coli* strain hosting the gfp-tagged plasmid pKJK5 as the donor, and a natural lake microbial community as recipients. MPs from virgin PHBV, virgin PLA and commercial PLA were selected with chitosan serving as a natural control. The gene transfer frequency was evaluated both on the surface of MPs and in the liquid suspension phases by flow cytometry. There was no difference in HGT between virgin and commercial PLA. However, PHBV showed significantly higher gene transfer frequency than both types of PLA (virgin and commercial) and the control. *Pseudomonas*, *Aeromonas* and *Sphingomonas* were the dominant bacterial groups in the lake which took up the plasmid on the surface of these MPs. These findings highlight the need of a regulatory framework to assess and manage the environmental risk with the use of biodegradable plastics. This work received funding from the European Union's Horizon 2020 Research and Innovation programme, under

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1.10.P-Tu098 Microplastic Pollution: A Hidden Driver of Antibiotic Resistance Gene Transfer?

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Microplastics (MPs) have been detected globally, from beaches and ocean depths to air and sediment pollution. The widespread use of antibiotics in treating bacterial infections has increased their presence as emerging contaminants in aquatic environments, as wastewater treatment systems often fail to remove them. Antibiotics add selective pressure to bacteria, promoting antibiotic resistance and contributing to infections that undermine modern medicine. Microplastics, due to their small size, large surface area, hydrophobicity, and low degradability, serve as vectors for microbial colonization and biofilm formation. Compared to natural materials like rocks and leaves, MPs have a greater ability to support microbial communities. Additionally, MPs can adsorb environmental contaminants, including antibiotics, which makes biofilms on MPs hotspots for horizontal gene transfer. This process facilitates the spread of antibiotic resistance genes (ARGs) to potentially pathogenic bacterial species within microbial communities. Bacteria can exchange resistance genes easily via horizontal gene transfer, a process that is enhanced in biofilms due to the proximity of cells. We investigated how varying concentrations of polyvinyl chloride (PVC) and polystyrene (PS) MPs affect the frequency of ARG transfer in laboratory conditions. Furthermore, we explored how different MPs influence biofilm formation and ARG transfer efficiency. *Escherichia coli* cultures were grown in lysogeny broth (LB) medium, and MPs were added at concentrations ranging from 0 to 800 mg/L. After 24 and 48 hours, particles were collected, and bacteria attached to the particles were cultured on plates. MPs were characterized using dynamic light scattering (DLS) and scanning electron microscopy (SEM) to study their effect on biofilm growth. Surface roughness of MPs was found to influence biofilm formation, and the efficiency of antibiotic gene transfer varied significantly with the type of MPs. Compared to the control group without MPs, biofilms formed on MP surfaces significantly enhanced bacterial survival rates and increased the efficiency of ARG transfer. Future research will include field sampling to study ARG transfer dynamics in natural aquatic environments, offering insights into how MPs contribute to the spread of antibiotic resistance in real-world conditions.

1.10.P-Tu099 Chironomids as Vectors for Microplastic Transfer Across Ecosystems

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The transfer of microplastics (MPs) from aquatic to terrestrial ecosystems represents an emerging pathway of contamination with significant ecological implications. Chironomids (*Chironomus riparius*), which undergo aquatic larval development before emerging as terrestrial adults, serve as key vectors for MP transfer. This study investigated the ingestion, retention, and expulsion of MPs in *C. riparius* exposed to a complex mixture of polyethylene (PE), polyvinyl chloride (PVC), and polyamide (PA) particles at environmentally relevant concentrations. Using an OECD-compliant chronic exposure model, we monitored life-history traits and quantified MPs in both emerging adults and exuviae to assess the efficiency of particle transfer across life stages. Results revealed that MPs were consistently transferred from the aquatic larval stage to terrestrial adults, confirming *C. riparius* as a conduit for MP dispersal. However, exuviae contained significantly higher concentrations of MPs than adult specimens, indicating molting as a potential detoxification pathway. The use of a mixed MP composition in this study provides critical insights into the behavior of diverse polymers in sediment-rich environments, reflecting real-world contamination scenarios. These findings highlight the dual role of chironomids as both victims and vectors of MP pollution, emphasizing their importance in understanding the cross-ecosystem impacts of microplastics. This research underscores the need for comprehensive assessments of MP transfer dynamics in benthic invertebrates to better predict their ecological consequences and inform management strategies for contaminated habitats. This research was conducted as part of the Plastic Underground Integrated Cross-Sectoral Solutions to Micro and Nanoplastic Pollution in Soil and Groundwater Ecosystems project HORIZON-MSCA-2021-DN, funded by the European Union's Horizon Europe program under Grant Agreement No. 101072777

1.10.P-Tu100 Natural Particles to be used as Reference for the Risk Assessment of Particulate Pollutants

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Due to the increasing entry of microparticles into the environment, sensitivity to the health risk of particles in air, water and soil has increased significantly over the last two decades, both in society and in science. There is now no question that microparticles can also have a harmful effect on living beings through specific mechanisms. However, this environmental risk must be assessed using appropriate methods tailored to these mechanisms. The ecotoxicological effect and exposure assessment of particulate pollutants involves significantly more uncertainty factors than that of dissolved chemicals, which makes reliable risk assessment difficult. The potential mechanisms of action of the microparticles are often not clear, involving combined direct chemical and mechanical as well as indirect effects (e.g. feed dilution; binding of nutrients) that are difficult to separate. Since organisms in their natural environment are also exposed to organic and mineral microparticles that are similar to particulate pollutants introduced by humans in terms of their physical and chemical properties as well as their ecotoxicological behavior, it is important to distinguish pollutant-specific effects from general particle effects. Through increased knowledge about the effectiveness or effect-relevant properties of natural particles and the use of application-specific, tailor-made natural reference particles in ecotoxicological tests, specific effects of particulate pollutants can be identified. With this poster, we want to present a research project (NatuReP), where natural microparticles, which are similar in their morphological and physical properties to selected particulate pollutants, are to be examined with regard to their ecotoxicological behavior in various environmental matrices (water, sediment, soil) and with regard to their suitability as reference particles for the ecotoxicological assessment of particulate pollutants. To this end, we apply (1) state-of-the-art analytical methods for characterizing microparticles (chemical, physical, transport), (2) a wide range of ecotoxicological test systems (with organisms of different organizational and trophic levels), (3) and suitable statistical methods for correlating particle properties and -effect. The aim of this project is to identify naturally occurring particles to serve as tailored reference which can be recommended for use in the environmental risk assessment of particulate pollutants in practice. This project is funded by the German Federal Environmental Foundation (DBU 38446-01)

1.11 Unraveling the Complexities of PFAS From Environmental Toxicology to Human Health

1.11.T-01 A tale of Banned and Novel PFAS: Approach to Developmental Toxicity in Zebrafish Embryo

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The persistence, bioaccumulation, and detrimental effects to the environment and human health of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have led to their global ban, prompting the introduction of novel PFAS (perfluoroalkyl substances) alternatives. The most predominant among the PFAS alternatives are the perfluoroether carboxylic acids (PFECA) and perfluoroether sulfonic acids (PFESA). However, the environmental health and toxicity concerns associated with the PFECA and PFESA are largely unexplored. In this study, the zebrafish (*Danio rerio*) was utilised as a toxicological model to investigate the developmental toxicity of representatives of PFECAs, such as hexafluoropropylene oxide trimer acid (HFPO-TA) and perfluoro-3,6,9 trioxadecanoic acid (PFO3DA), and PFESAs, such as perfluoro-3,6-dioxo-4-methyl-7-octenesulfonic acid (NBP1) and 7H-perfluoro-4-methyl-3,6-dioxaoctanesulfonic acid (NBP2), alongside the most widely studied and banned PFAS, PFOA, and PFOS potassium salt (PFOS-K). We assessed the acute toxicity by calculating the median lethal concentration (LC50) in zebrafish embryos exposed to the six previously mentioned PFASs at concentrations ranging from 0 to 100 mg/L. Additionally, developmental anomalies, hatching rate, heart rate, and body length were evaluated under exposure to environmentally relevant PFAS concentrations (7, 70, 350, and 700 µg/L) in the period from 6 and 96 hours post-fertilisation (hpf). Results showed that the LC50 values of PFOA (>100 mg/L) and PFOS-K (54.56 mg/L) aligned with earlier studies. Likewise, the calculated LC50 values show the hierarchy of toxic potency as follows: NBP1 > PFOS-K > PFO3DA > NBP2 > HFPO-TA > PFOA. Furthermore, PFASs exposure at environmental concentrations resulted in a diverse occurrence of developmental malformations, including pericardial oedema, uninflated swim bladder, notochord malformation, blood congestion, yolk sac oedema/deformation, impaired fin development, and short body length, among others at 96 hpf. The heart rate was significantly inhibited except for PFOA and NBP1, which caused a significant increase in heart rate. Meanwhile, the body length of the larva was significantly reduced across the tested PFASs. In summary, the novel PFAS alternatives

may induce developmental toxicity in zebrafish embryos, suggesting that they are not safer substitutes for PFOS and PFOA. This project has received funding from the European Union's Horizon-MSCA-2022-PF-01 under the Marie Skłodowska-Curie grant agreement No. [101108489].

1.11.T-02 Upscaling PFAS Dynamics from Trophic Chains to Ecosystem Responses in an AFFF Contaminated Environment

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Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals renowned for their persistence, bioaccumulation, and ecological risks. This study investigates PFAS contamination at a firefighting training site in Trelleborg, Sweden, analyzing 158 environmental and biological samples spanning terrestrial and aquatic ecosystems. Through stable isotope analysis (SIA) to reconstruct trophic interactions and LC-MS/MS quantification of 16 PFAS, bioaccumulation and biomagnification dynamics were assessed. Results reveal that PFOA and PFOS dominate contamination profiles due to their historical use in aqueous film-forming foams (AFFF). Bioconcentration and bioaccumulation factors highlight higher PFAS accumulation in aquatic systems, with biomagnification observed for PFOA and PFOS. In terrestrial ecosystems, short-chain congeners such as PFHxA and PFHpA consistently demonstrate biomagnification trends. This study highlights that short-chain PFAS, once considered less bioaccumulative, can significantly accumulate and even biomagnify in terrestrial food webs. These findings challenge conventional assumptions about their safety as alternatives to long-chain PFAS and underscore their potential ecological risks. This work emphasizes the necessity of integrating short-chain PFAS into environmental risk assessments and adopting stricter regulatory measures to mitigate their impact. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101037509 (SCENARIOS project)

1.11.T-03 Blood-based Lipid Perturbations Linked to Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water and Occupational Exposures

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Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic, persistent chemicals associated with health concerns, particularly in communities near production and release sites. Firefighters are also often exposed to PFAS through protective turnout gear and aqueous film-forming foam applications. PFAS have been implicated with increased cholesterol in humans, but other lipid species have not been evaluated extensively. In this study, we explore both PFAS presence and lipid changes in human serum for two cohorts, one with high drinking water exposure (DW, n=49) and the other having exposure through their firefighting occupation (FF, n=29). Specifically, a non-targeted analysis platform coupling liquid chromatography, ion mobility spectrometry, collision induced dissociation, and mass spectrometry (LC-IMS-CID-MS) separations was used to evaluate >100 PFAS and >800 lipid species. PFAS and lipids were extracted separately from the serum samples prior to instrumental analysis. Data was then annotated using Skyline software and multidimensional in-house libraries containing LC, IMS, CID, and MS information. Fourteen PFAS were also quantified to understand their levels in the two cohorts. In the quantitative analyses, PFAS concentrations were found to be significantly higher in the DW cohort than in the FFs. To assess lipid changes due to PFAS exposure, all study participants were grouped as either having ≥20 ng/mL serum (n=34) or <20 ng/mL (n=44) for the sum of 7 PFAS, based on the National Academies Consensus Study Report guidelines for serum PFAS concentrations expected to have health impacts. Notably, 33 of the 34 samples in the high group were from the DW cohort. In the serum lipidomic analyses, 388 unique lipids from 15 classes were identified. Of these, 23 lipids were found to be statistically significant in the ≥20 ng/mL group, with 22 being upregulated, and phosphatidylethanolamines (PEs) and triglycerides (TGs) comprising 60.8% of the significant lipids. While TGs have known associations with PFAS exposures, PEs are newly implicated in PFAS-related dyslipidemia. This is important, as PEs are the second most abundant glycerophospholipid in mammalian cells, following phosphatidylcholines (PCs) and abnormal PC/PE ratios have been linked to an array of

liver diseases and reduced liver regeneration. These lipid changes should encourage further exploration of PFAS toxicity in humans and assist in informing regulatory limits.

1.11.T-04 Next Generation Risk Assessment of Poly- and Perfluoroalkyl Substances through Bayesian Network based Quantitative Adverse Outcome Pathway

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Poly- and perfluoroalkyl substances (PFAS) have recently been linked to potential neurotoxic effects, raising human health concerns. However, with thousands of PFAS compounds in existence, the traditional regulatory approaches face significant challenges addressing their potential risks. Thus, this study aims to evaluate the neurotoxic risks of PFAS using a Next Generation Risk Assessment (NGRA) framework that integrates New Approach Methodologies (NAMs) and an Adverse Outcome Pathway (AOP) framework with Bayesian Network (BN) modeling. The workflow consisted of four key steps. First, in vivo data from regulatory reports and in vitro data from literature, high-throughput screening (HTS) database, and in-house experiments were collected. Second, a neurotoxicity AOP was constructed using the AOP Wiki, with data assigned to specific molecular initiating events (MIEs), key events (KEs), and adverse outcomes (AOs). Third, a BN model was trained to calculate the probabilities of activation for each event, enabling a probabilistic assessment of PFAS risk. Finally, human exposure levels and effect distribution were incorporated to quantitatively evaluate the neurotoxicity risk of PFAS in humans. The results revealed that PFAS acts as an antagonist to thyroid receptors at the MIE level, disrupting signaling pathways and triggering significant activation across all KEs, leading to cell death. At the AO level, these effects manifested as neurotoxicity, including degenerative diseases and cognitive impairments. Probabilistic analysis using the BN model demonstrated a 24% likelihood of reduced cell viability at PFAS concentrations of 100 300 μ M. The integration of in vivo and in vitro data provided a comprehensive and quantitative evaluation of PFAS-induced neurotoxicity. And, the risk assessment incorporating human exposure values did not reveal any significant effects. In conclusion, a BN model based quantitative AOP was utilized to evaluate the neurotoxic risks of PFAS by integrating in vivo, in vitro data. This study contributes to the advancement of NGRA approaches by providing a robust framework for assessing the risks of PFAS. This work was supported by Korea Environmental Industry & Technology Institute (KEITI) through Technology Development Project for Safety Management of Household Chemical Products (RS-2023-00215309), funded by Korea Ministry of Environment (MOE).

1.11.P-Tu103 Toxicogenomic Assessment of PFAS in Modified Zebrafish Embryo Test (FET) and Immune-Challenged Assays

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Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals that are widely used in industrial applications and consumer products due to their durability and resistance to water, oil, and heat. However, their environmental persistence and bioaccumulation in living organisms raise serious concerns about their potential health effects and immunotoxicity. Despite the widespread exposure to PFAS, there is a significant gap in understanding the molecular mechanisms that drive their toxicity, particularly in relation to immunotoxicity. The primary goal of this study is to address this gap by using advanced OMIC technologies to investigate the molecular effects of PFAS exposure in a relevant model organism, zebrafish. Specifically, we aim to examine the toxicological effects of PFOA, a well-known PFAS, and GenX, a PFOA substitute, through gene expression analysis. We will employ the Fish Embryo Toxicity Test (FET) in its conventional form and a modified version that incorporates an immune challenge to assess the impact of PFAS on the immune system. In the conventional FET, zebrafish embryos are exposed to PFAS for 96 hours, followed by RNA sequencing to identify differentially expressed genes (DEGs) linked to toxicity. In the modified FET, pathogen-associated molecular patterns (PAMPs) are introduced to stimulate the immune response, allowing us to evaluate potential immunotoxicity. The resulting data will be analyzed using various bioinformatic tools, including Venn diagrams and heatmaps, to better understand the molecular pathways affected by PFAS exposure. Although results are still being generated, the study is expected to provide valuable insights into the gene expression changes associated with PFAS exposure and contribute to the identification of biomarkers for general toxicity and immunotoxicity. These findings will significantly advance our understanding of PFAS toxicity, especially in the context of immune system disruption, and will help fill a crucial gap in the current toxicological data. This research is highly relevant for advancing the scientific knowledge on PFAS toxicity, which can inform future public health guidelines and policies aimed at reducing PFAS exposure and its associated risks. The goal is to apply this test design to future PFAS alternatives as well, enabling a faster and more

efficient ecotoxicological risk assessment, as PFAS compounds are expected to be gradually replaced over time due to their toxicity.

1.11.P-Tu104 Major Alterations in Guppy Reproductive Traits Induced by PFOA and GenX Environmental Concentrations

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In humans and rodents, exposure to per- and poly-fluoroalkyl substances (PFAS) has been associated with negative reproductive health effects. However, our understanding of their impact on reproduction in aquatic organisms is still limited. To address this knowledge gap, we exposed adult freshwater fish (*Poecilia reticulata*) to 1 µg/L of perfluorooctanoic acid (PFOA), a legacy PFAS, or its alternative, the ammonium salt of hexafluoropropylene oxide dimer acid (HFPO-DA; trademark name: GenX), which is considered potentially less hazardous. We assessed PFAS bioaccumulation in whole-body fish and key reproductive traits such as levels of vitellogenin and sexual hormones, sperm quantity and quality, male sexual activity, mating tactic, and colouration, after 90 days of exposure. Moreover, in the 5-months offspring (F1), exposed during pregnancy (*P. reticulata* is a live-bearing fish) and moved to clean water till reaching maturity, we measured fertility, sperm quantity and quality, and behaviour (boldness). General health conditions (e.g. swim performance, mortality) were also evaluated. Although no clear effects on survival and overall health were observed, sublethal effects were reported. We demonstrated that PFOA and GenX significantly impaired key traits for reproductive success. Levels of estradiol and testosterone were altered in both sexes; moreover, the exposed males performed less courtship behaviour but increased the number of sneak mating attempts, particularly when exposed to PFOA. We discovered that males prenatally exposed to PFOA and GenX presented a decrease in body colouration, a proxy of health status and reproductive success; in addition, PFOA inhibited fish risk-taking behaviour. Finally, detectable amounts of PFOA were found either in F0 directly exposed fish than in F1 adult fish exposed during the prenatal stage (<0.5 µg/g). This study offered a comprehensive comparison of two PFAS and shed light on the toxicity of an emerging PFOA alternative, GenX. Overall, we showed that even at low concentrations GenX, but mostly PFOA, can have subtle but significant harmful effects. These results improve the knowledge on the effects PFAS might have on reproduction, and provide important data for the research on PFAS toxicity in humans. From an environmental risk assessment perspective, these results emphasize the potential ramifications of pollution under natural conditions and their far-reaching consequences for fish populations and ecosystems. This work was supported by the University of Padova under the funding scheme STARS@UNIPD2021 (Prot. no. 3091/2022), and by the Italian Ministry of University and Research (MUR) under the funding scheme Research Projects of National Relevant Interest (Prot. no. 20228ECFT2).

1.11.P-Tu123 Discrepancies between CCK-8 and Trypan Blue Assays in PFAS-Exposed ZF4 Cells: Implications for Metabolic Stress Assessment

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Perfluoroalkyl substances (PFAS), particularly perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), are persistent environmental contaminants with potential toxicological effects. This study investigated the effects of PFOA and PFOS on zebrafish embryonic fibroblast (ZF4) cells, revealing critical methodological considerations for cytotoxicity assessments. ZF4 cells were exposed to various concentrations of PFOA and PFOS (100-1000 µM) for 24 hours. Cell viability was evaluated using the cell counting kit 8 (CCK-8) assay, trypan blue cell counting, and lactate dehydrogenase (LDH) assay, which measures cell membrane damage and LDH leakage from the cytoplasm. Surprisingly, the CCK-8 assay showed a dose-dependent increase in absorbance readings at concentrations up to 500 µM, particularly for PFOS, suggesting increased cell viability or proliferation. However, concurrent trypan blue cell counts revealed a dose-dependent decrease in cell numbers, with IC50 values determined for both PFOA and PFOS (570.1 µM and 586.1 µM, respectively). LDH assay results were consistent with trypan blue counts. This discrepancy between CCK-8 results and actual cell counts indicates that the CCK-8 assay may produce false-positive results in this context. The increased CCK-8 signal, despite reduced cell numbers, suggests that PFOA and PFOS exposure may induce metabolic stress in ZF4 cells, potentially leading to increased mitochondrial activity. These findings highlight the importance of using multiple

assays to assess cell viability and stress responses, especially when studying PFAS toxicity in emerging model cell lines like ZF4. Furthermore, this research suggests the potential value of investigating mitochondrial bioenergetics in PFAS-exposed cells, as metabolic alterations may precede overt cytotoxicity. To further confirm enhanced metabolic stress, future work will include Seahorse experiments to measure cellular respiration and glycolysis. Additionally, mitochondrial functional studies will be conducted using flow cytometry to analyze MitoSOX and TMRM staining, providing insights into mitochondrial superoxide production and membrane potential, respectively. These results contribute to our understanding of PFAS toxicity mechanisms and provide critical methodological considerations for future toxicological studies, emphasizing the need for multiple assays in assessing cell viability and stress responses, particularly when studying emerging model cell lines. The authors gratefully acknowledge the Petroleum Technology Development Fund (PTDF) for awarding a Ph.D. scholarship to Timothy Prince Chidike Ezeorba (PTDF Scholar ID: 22PHD053). We also extend our appreciation to the Society of Environmental Toxicology and Chemistry (SETAC) Europe for providing a Student Registration Grant, enabling the presentation of our findings at the 2025 SETAC Europe Annual Meeting.

1.11.P Unraveling the Complexities of PFAS From Environmental Toxicology to Human Health

1.11.P-Tu101 Effects of Flame Retardants on Survival, Reproduction, Energy Metabolism, and Transcriptional Modulation of Brackish Water Flea *Diaphanosoma celebensis*

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Marine plastic pollution has become a major environmental issue worldwide. Plastic products contain harmful chemicals, and because these chemicals can leach into media, they are considered an emerging contaminant in ocean. Flame retardants (FRs) are one of the commonly used additives in plastic industries to enhance products flame resistance. As the use of some brominated FRs (BFRs) have globally banned due to their high ecotoxicity, organophosphate FRs (OPFRs) have recently been proposed as alternatives to BFRs, but information on their toxic effects on marine ecosystem is still lacking. Thus, in this study, we investigated toxic effects of OPFRs [triphenyl phosphate, TPhP; Tris(1,3-dichloro-2-propyl) phosphate, TDCPP], together with representative BFR, hexabromocyclododecanes (HBCDs) on survival, reproduction, energy metabolism of brackish water flea *Diaphanosoma celebensis*. Subsequently, we further investigated toxic mechanisms of TPhP through transcriptomic analysis. Acute toxicity test showed that both OPFRs were acutely toxic to *D. celebensis* (48-h LC₅₀ of TPhP and TDCPP: 2.41 and 17.12 mg/L, respectively), but HBCDs did not induce significant mortality up to 20 mg/L. Chronic toxicity test showed that all FRs caused reproductive disorder through reducing fecundity even at the sub-lethal concentrations, but only TPhP negatively affected lifespan. The contents of energy reserves (carbohydrate, protein, and lipid) were differently modulated following exposure to FRs. Transcriptomic analysis showed that genes related to energy metabolism-related pathways including metabolic pathways, AMPK signaling pathway, fatty acid biosynthesis, fatty acid degradation, and metabolism of various amino acids were mainly affected following TPhP exposure. In addition, genes related to nervous system, immune system, and genetic modulation were significantly modulated by TPhP. Our findings showed high toxicity of OPFRs as well as BFRs and suggest that OPFRs, especially TPhP, have potential toxicity to disturb energy metabolism, reproduction, and various biological processes. This study provides a better understanding of the molecular mode of action of FRs in marine zooplankton. This research was supported by the Risk assessment to prepare standards for protecting marine ecosystem of the Korea Institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (KIMST-20220383).

1.11.P-Tu102 Investigating In-vivo Toxicity of PFAS on the Development of Zebrafish Embryos as a Model for Environmental Impact

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Per- and polyfluoroalkyl substances (PFAS) represent a broad class of over 10,000 synthetic organic chemicals, recognized for their widespread applications as surfactants and repellents. The rapid pace of industrialization and urbanization in India has intensified concerns regarding the extensive use of PFAS across multiple sectors, including textiles, personal care products, non-stick cookware, electronics, and firefighting foams and gear. PFAS have been detected in various environmental media, including air, water, wastewater, soil, human blood, hair, and breast milk, as well as in biota, where they bioaccumulate within the food chain due to their persistent nature and resistance to degradation. Consequently, PFAS

contamination in water sources poses potential health risks to millions of people through drinking water supplies. The widespread presence of PFAS necessitates a comprehensive toxicity assessment to elucidate their adverse health impacts, yet there remains a scarcity of toxicity data for most PFAS used in industrial and consumer products. The zebrafish (*Danio rerio*) is emerging as a robust animal model for rapid toxicity assessment, offering predictive insights into potential human health hazards. Zebrafish possess morphological and molecular features in their tissues and organs that are either identical or highly analogous to those of other vertebrates, including humans, making them ideal for evaluating PFAS toxicity. This study investigates the developmental effects of exposure to 24 PFASs comprising long-chain and short-chain PFAS and their precursors on zebrafish embryos. The compounds include C4 C14 perfluorocarboxylic acids (PFCAs), C4 C10 perfluorosulfonic acids (PFSAs), perfluorooctanesulfonamide (FOSA), N-methyl perfluorooctanesulfonamidoacetic acid (N-MeFOSAA), N-ethyl perfluorooctanesulfonamidoacetic acid (N-EtFOSAA), and 4:2, 6:2, and 8:2 fluorotelomer sulfonates (FTS). The study examines morphological deformities (spinal cord curvature, body length, head/eye area, head and trunk angle), hatching period, survival rate, heart rate, blood flow rate, and stress responses using ImageJ and histopathological analysis for cellular-level damage. This work provides critical insights into PFAS-associated developmental toxicity, aiding in risk assessment and informing regulatory policies on PFAS exposure limits.

1.11.P-Tu103 Toxicogenomic Assessment of PFAS in Modified Zebrafish Embryo Test (FET) and Immune-Challenged Assays

Tim Benad¹, Sebastian Eilebrecht², Uwa Steve Ayobahan¹ and Bernd Gockener¹, (1)*Fraunhofer Institute for Molecular Biology and Applied Ecology, Germany,* (2)*Ecotoxicogenomics, Fraunhofer Institute for Molecular Biology and Applied Ecology, Germany*

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals that are widely used in industrial applications and consumer products due to their durability and resistance to water, oil, and heat. However, their environmental persistence and bioaccumulation in living organisms raise serious concerns about their potential health effects and immunotoxicity. Despite the widespread exposure to PFAS, there is a significant gap in understanding the molecular mechanisms that drive their toxicity, particularly in relation to immunotoxicity. The primary goal of this study is to address this gap by using advanced OMIC technologies to investigate the molecular effects of PFAS exposure in a relevant model organism, zebrafish. Specifically, we aim to examine the toxicological effects of PFOA, a well-known PFAS, and GenX, a PFOA substitute, through gene expression analysis. We will employ the Fish Embryo Toxicity Test (FET) in its conventional form and a modified version that incorporates an immune challenge to assess the impact of PFAS on the immune system. In the conventional FET, zebrafish embryos are exposed to PFAS for 96 hours, followed by RNA sequencing to identify differentially expressed genes (DEGs) linked to toxicity. In the modified FET, pathogen-associated molecular patterns (PAMPs) are introduced to stimulate the immune response, allowing us to evaluate potential immunotoxicity. The resulting data will be analyzed using various bioinformatic tools, including Venn diagrams and heatmaps, to better understand the molecular pathways affected by PFAS exposure. Although results are still being generated, the study is expected to provide valuable insights into the gene expression changes associated with PFAS exposure and contribute to the identification of biomarkers for general toxicity and immunotoxicity. These findings will significantly advance our understanding of PFAS toxicity, especially in the context of immune system disruption, and will help fill a crucial gap in the current toxicological data. This research is highly relevant for advancing the scientific knowledge on PFAS toxicity, which can inform future public health guidelines and policies aimed at reducing PFAS exposure and its associated risks. The goal is to apply this test design to future PFAS alternatives as well, enabling a faster and more efficient ecotoxicological risk assessment, as PFAS compounds are expected to be gradually replaced over time due to their toxicity.

1.11.P-Tu104 Major Alterations in Guppy Reproductive Traits Induced by PFOA and GenX Environmental Concentrations

Marianna Pauletto¹, Edoardo Pietropoli¹, Alessandro Devigili², Silvia Iori¹, Francesco Santi³, Francesco Eugenio Leonardi³, Lorena Lucatello¹, Mery Giantin¹, Andrea Barbarossa⁴, Anisa Bardhi⁴, Francesca Capolongo⁵, Mauro Dacasto⁵ and Clelia Gasparini³, (1)*University of Padova - Department of Comparative Biomedicine & Food Science, Italy,* (2)*Univeristy of Padova - Department of Biology, Italy,* (3)*University of Padova - Department of Biology, Italy,* (4)*University of Bologna - Department of Veterinary Medical Sciences, Italy,* (5)*University of Padova - Department of Comparative Biomedicine and Food Science, Italy*

In humans and rodents, exposure to per- and poly-fluoroalkyl substances (PFAS) has been associated with negative reproductive health effects. However, our understanding of their impact on reproduction in

aquatic organisms is still limited. To address this knowledge gap, we exposed adult freshwater fish (*Poecilia reticulata*) to 1 µg/L of perfluorooctanoic acid (PFOA), a legacy PFAS, or its alternative, the ammonium salt of hexafluoropropylene oxide dimer acid (HFPO-DA; trademark name: GenX), which is considered potentially less hazardous. We assessed PFAS bioaccumulation in whole-body fish and key reproductive traits such as levels of vitellogenin and sexual hormones, sperm quantity and quality, male sexual activity, mating tactic, and colouration, after 90 days of exposure. Moreover, in the 5-months offspring (F1), exposed during pregnancy (*P. reticulata* is a live-bearing fish) and moved to clean water till reaching maturity, we measured fertility, sperm quantity and quality, and behaviour (boldness). General health conditions (e.g. swim performance, mortality) were also evaluated. Although no clear effects on survival and overall health were observed, sublethal effects were reported. We demonstrated that PFOA and GenX significantly impaired key traits for reproductive success. Levels of estradiol and testosterone were altered in both sexes; moreover, the exposed males performed less courtship behaviour but increased the number of sneak mating attempts, particularly when exposed to PFOA. We discovered that males prenatally exposed to PFOA and GenX presented a decrease in body colouration, a proxy of health status and reproductive success; in addition, PFOA inhibited fish risk-taking behaviour. Finally, detectable amounts of PFOA were found either in F0 directly exposed fish than in F1 adult fish exposed during the prenatal stage (<0.5 µg/g).

This study offered a comprehensive comparison of two PFAS and shed light on the toxicity of an emerging PFOA alternative, GenX. Overall, we showed that even at low concentrations GenX, but mostly PFOA, can have subtle but significant harmful effects. These results improve the knowledge on the effects PFAS might have on reproduction, and provide important data for the research on PFAS toxicity in humans. From an environmental risk assessment perspective, these results emphasize the potential ramifications of pollution under natural conditions and their far-reaching consequences for fish populations and ecosystems. This work was supported by the University of Padova under the funding scheme STARS@UNIPD2021 (Prot. no. 3091/2022), and by the Italian Ministry of University and Research (MUR) under the funding scheme Research Projects of National Relevant Interest (Prot. no. 20228ECFT2).

1.11.P-Tu105 Hemolytic Activity and Proteomic Insights into PFAS Toxicity in *Eisenia fetida*

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The evaluation of emerging and legacy contaminants remains a central focus in ecotoxicological research and environmental safety assessment. This study investigates the effects of 34 per- and polyfluoroalkyl substances (PFAS), encompassing diverse chemical classes (legacy and emerging, short- and long-chain, carboxylates, sulfonates, ethers, and polyethers), on the hemolytic activity of cell-free coelomic fluid derived from *Eisenia fetida*. A combined experimental framework involving in vivo and in vitro exposures was implemented, supplemented by advanced proteomic analyses performed on hemolymph samples using liquid chromatography coupled with mass spectrometry (LC-MS) to quantify hemolysin content and investigate molecular alterations induced by PFAS exposure. In vivo exposures were conducted in adherence to the OECD 207 acute toxicity test, employing PFAS concentrations of 0.6 µM and 229 µM. For in vitro studies, cell-free coelomic fluid was incubated with PFAS at (sub)nanomolar concentrations. Following these exposures, hemolytic activity was assessed using a refined sheep erythrocyte hemolysis assay. Methodological advancements included precise quantification of erythrocyte survival rates and the establishment of a standardized dilution curve for coelomic fluid, ensuring consistent and reproducible measurements. Preliminary findings indicate statistically significant differences in hemolytic activity between controls and PFAS-treated groups, with reduced hemolysis associated with specific chemical subclasses. Proteomic data further identified modifications in hemolysin and other proteins such as CCF-1 related with immune defense mechanisms. This comprehensive approach provides critical insights into the molecular mechanisms underlying PFAS toxicity in non-model organisms, paving the way for the development of sensitive and predictive methodologies for the environmental risk assessment of these pervasive contaminants. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101037509 (SCENARIOS project)

1.11.P-Tu106 Modulatory Impact of PFAS on GABA Receptor-Mediated Currents in Neuron-like Cells

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants that have raised concerns due to their widespread distribution and adverse effects on biological processes. Their high stability enables these compounds to accumulate in the environment and living organisms, where they may disrupt critical physiological functions, including hormonal balance, immune function, and neurological and developmental pathways. The pervasive nature and extensive biological impact of PFAS underscore the need for strict regulatory actions and ongoing research to mitigate their harmful effects. In particular, concerns are growing over the neurotoxic potential of PFAS. Although evidence increasingly suggests these chemicals harm the nervous system, the exact mechanisms remain under investigation. This study examines how different PFAS congeners affect gamma-aminobutyric acid (GABA) receptor-mediated currents in differentiated S1 neuroblastoma cells, which exhibit neuron-like characteristics. Using patch clamp electrophysiology, we confirmed the presence of GABA-evoked currents, and PFAS exposure significantly and reversibly reduced these currents. We also conducted molecular docking studies to evaluate PFAS interactions with GABA receptors; those with the highest affinity were selected for further cytotoxicity testing. Additionally, STAB1 neuroblastoma cells were exposed to various concentrations of short-chain PFAS, legacy PFAS, and mono- or polyether PFAS, exhibiting a hormetic response, with cell proliferation at lower concentrations and cytotoxicity at higher concentrations. Our findings show that PFAS can significantly reduce GABA-evoked current amplitude, suggesting disruption of GABA receptor function and contributing to their neurotoxic properties. These results provide the first direct evidence of PFAS impairing GABAergic neurotransmission in neuron-like cells, highlighting the need for further research into their neurological impacts. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101037509 (SCENARIOS project)

1.11.P-Tu107 PFAS and Nanoplastics Co-Exposure Impacts on Human Intestinal Cells: Combining Metabolomics and Phenomics

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Per- and polyfluoroalkyl substances (PFAS) and nanoplastics (NPs) pose significant environmental and health challenges due to their persistence, bioaccumulation potential, and toxicity. While both pollutants are individually known to disrupt metabolic and phenotypic processes, their combined impacts on human gut health remain poorly understood. This study investigated the co-exposure effects of PFAS, specifically perfluorooctanesulfonic acid (PFOS), and polystyrene NPs on human intestinal Caco-2 cells using integrated metabolomics and phenomics approach. Caco-2 cells were exposed to PFOS, NPs, or their combination, and phenotypic alterations were assessed using the Cell Painting assay, a high-content imaging assay coupled with automated image analysis. Metabolic and lipidomic changes were analyzed through ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOFMS). Phenotypic profiling revealed that co-exposure to PFOS and NPs caused more pronounced effects on subcellular structures, including the F-actin cytoskeleton, Golgi apparatus, and plasma membrane, compared to single exposures. Interestingly, mitochondrial structure was largely affected by NPs alone. Correlation analysis supported a relationship between PFOS and NPs with co-exposure phenotype, indicating shared mechanisms of toxicity. Lipidomic profiling demonstrated that NPs alone induced changes of cellular lipids, while PFOS exposure primarily altered polar metabolite profiles. PFOS alone or under co-exposure revealed changes in carnitines, involved in mitochondrial fatty acid β -oxidation pathways. The distinct and overlapping impacts of PFOS and NPs on cellular phenotypes and metabolic pathways underscore the complexity of their combined toxicity. This study emphasizes the need for advanced integrative approaches, such as phenomics and metabolomics, to unravel the mechanisms underlying co-exposures to PFAS and nanoplastics. By providing insights into the phenotypic and metabolic disruptions caused by PFOS and NP co-exposure, this work contributes to a better understanding of their combined health risks and supports the development of mechanism-based risk assessment strategies.

1.11.P-Tu108 Evaluating PFAS-Related Human Health and Environmental Risks Before and After the Implementation of the LIFE SOuRCE Solution

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LIFE SOuRCE is a European project focusing on the demonstration and evaluation of sustainable on-site remediation technologies for PFAS-contaminated groundwater. Four different techniques are combined and evaluated as treatment trains, called the LIFE SOuRCE solutions. This study aims to evaluate the human health and environmental risks associated with PFAS in groundwater before and after the implementation of the LIFE SOuRCE solutions. The assessment focuses on PFAS exposure risks to human health and environmental impacts on a river ecosystem. Two sites with different risk profiles are analysed, 1) an industrial and agricultural area in Spain for Human Health Risk Assessment (HHRA) and 2) a Swedish river ecosystem for Environmental Risk Assessment (ERA). For HHRA, two exposure scenarios were evaluated at the Spanish site. Scenario 1 involves industrial workers exposed to PFAS-contaminated water during cleaning tasks, evaluating ingestion and dermal contact. Scenario 2 involves farmers using groundwater for crop irrigation and consumers potentially ingesting PFAS through these crops. Lettuce was selected as the test crop. Exposure pathways considered include ingestion and dermal contact for farmers, and ingestion for consumers (adults and children) through PFAS transferred to crops. Baseline data, collected before the LIFE SOuRCE solution implementation, indicated acceptable carcinogenic risk levels (carcinogenic risk values $< 1 \times 10^{-5}$) but the systemic risk was above the admissible threshold (hazard index > 1) for both scenarios, due to the presence of PFBA, PFDoDA, PFHpA, PFOS, PFOA, PFPeA and PFUDA. For ERA, PFAS risk to the Swedish river ecosystem and fish populations was assessed, establishing a baseline Toxicity Unit (TU) risk prior to the LIFE SOuRCE solution. Predicted Environmental Concentrations (PEC) were calculated using the highest PFAS concentrations found, and environmental risk was assessed against Predicted No-Effect Concentrations (PNEC) for aquatic organisms, obtained from the NORMAN Ecotoxicology Database. Results showed most PFAS within acceptable levels ($TU < 1$); however, PFOS and PFOA concentrations at certain points suggest possible risk to aquatic organisms. Baseline HHRA and ERA results will allow for an evaluation of the effectiveness of PFAS mitigation following the implementation of the LIFE SOuRCE solution.

1.11.P-Tu109 In Silico Classification Model to Screen the Potential Thyroid Hormone System-Disrupting Activity of Per- and Polyfluoroalkyl Substances

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Per- and polyfluoroalkyl substances (PFAS) represent a broad class of man-made compounds characterised by highly stable carbon-fluorine bonds, well known for being persistent in the environment, bioaccumulative, and toxic for humans and wildlife. Several studies linked PFAS exposure to multiple biological mechanisms leading to adverse effects on living organisms, including their ability of disrupting the thyroid hormone (TH) system by competing with the TH thyroxine (T4) for binding with the human transthyretin (hTTR), a key TH distributor protein. This biological mechanism can modulate serum TH concentration resulting in severe TH system dysfunctions. To speed up the identification of hazardous compounds, including TH system-disrupting chemicals (THSDCs), international authorities promote the use of in silico methods (e.g. Quantitative Structure-Activity Relationship (QSAR) based approaches). This work aims to develop a new QSAR classification model, following the OECD principles, to predict the binding ability of PFAS with hTTR. A newly published dataset containing binding ability records of 136 structurally heterogeneous PFAS, measured with the 8-anilino-1-naphthalenesulfonic acid (ANSA) based binding in vitro assay, was selected for QSAR development through Linear Discriminant Analysis (LDA). After data curation, the remaining 119 PFAS were categorised into two classes according to their median % activity: 74 PFAS were categorised as strong hTTR binders (median % activity greater or equal to 50%), while 45 PFAS as weak or not hTTR binders (median % activity smaller than 50%). Harmonized SMILES (Simplified Molecular Input Line Entry System) of the structures were used as input for the calculation of simple theoretical molecular descriptors, which were used as variables in the QSAR modeling. For each class, PFAS were sorted by structure, then two every three PFAS were assigned to the training set for QSAR development, and the remaining compounds were assigned to the test set for QSAR external validation. The final model, calibrated using all the available experimental data, is characterised by accuracy, sensitivity, and specificity equal to 87%, 91%, and 80%, respectively. The proposed QSAR extends the applicability domain of previously published similar models for the prediction of the same endpoint, and can be applied as a simple tool for screening the potential thyroid hormone system-disrupting activity of PFAS.

1.11.P-Tu110 Does the Addition of Perfluoro-Moieties Impact Mechanisms of Toxic Action of Organic Chemicals? An In Silico Approach

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The PFAS exposure problem has become ubiquitous. The poor biodegradability of such substances in the

environment has been recognised for decades. This is an unfortunate environmental fate property but a huge benefit for certain applications (the most obvious example being the long-lasting, panphobe property facilitating use of PTFE as a non-stick polymer on saucepans and a PFAS emulsifier (PFOA) to carry it). Due to the multifaceted utility of perfluorinated compounds, industry has diligently placed >4000 PFAS on the market and the EU Commission has recently recognised the potential for forever chemicals to pose a threat to aquatic and terrestrial organisms alike. While much research has been carried out on the persistence of PFAS, and to a lesser extent bioaccumulation and fate, less is known about the mechanisms of toxic action of such compounds which can impact the aquatic environment. This poster explores the potential for perfluorination to enhance or reduce acute and chronic toxicity for algae, daphnids and fish. A set of internally validated experimental aquatic toxicity studies on PFAS were selected. Their Mechanisms of toxic Action (MechoA) were allocated using MechoA Premium and the experimental water solubility determined (from existing studies or by the iSafeRat® WATSOL module). The data were then plotted on the appropriate iSafeRat® aquatic toxicity QSAR according to the designated MechoA. The models excluded fluorinated substances. The positions of the PFAS points were then compared to the PFAS-free regression lines to ascertain whether excess or reduced toxicity was observed compared to the classical QSARs. Their distance to the regression was assessed statistically to evaluate if these compounds could be included to these model with confidence. Data were obtained for several MechoA including non-polar narcotics, polar narcotics, hydrolysis to non-polar narcotics product and hard electrophile reactivity. The substances were then classified excess toxicity, reduced toxicity or indifferent toxicity compared to the PFAS-free QSARs. Furthermore, as these substances tend to be rather difficult to test for both ecotoxicity and water solubility, NAMs will help elucidate the extent of the PFAS hazard profiles. The results are discussed in the poster.

1.11.P-Tu111 Scientific Basis for Guide Values for 11 PFASs in Soil

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Growing concerns about the health and environmental impacts of PFAS have prompted increased research and regulatory scrutiny. This study aimed at developing the scientific basis for guide values for 11 per- and polyfluoroalkyl substances (PFAS) in soil, with a focus on effects on soil fertility including the protection of biocenosis of organisms with soil direct contact. The study is part of a larger project initiated as a response to a Swiss parliamentary motion to set limit values for PFAS under different Swiss ordinances. A literature search revealed limited availability of effect data for PFAS on soil organisms, with most studies available for PFOS and PFOA. It was not possible to derive guideline values based on the assessment factor method for all 11 PFAS. Hence, the potential of using relative potency factors was explored. In addition to the direct toxicity to soil organisms secondary poisoning to earthworm eating birds and mammals has been assessed. The study acknowledges existing data limitations and emphasizes the need for further research on PFAS compounds beyond PFOS and PFOA. Overall, this research contributes to the development of evidence-based soil guide values for PFAS in Switzerland, addressing both direct and indirect ecological impacts.

1.11.P-Tu112 Transcriptomic Sensitivity of Acute and Chronic In Vitro Exposure to Perfluorooctanoic Acid in Human Vascular Endothelial Cells: Insights from Benchmark Concentration Modeling

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Perfluorooctanoic acid (PFOA) is a synthetic chemical in the class of per- and polyfluoroalkyl substances (PFAS), which have been widely synthesized and used in various industries and consumer products since the 1940s. Due to their extreme persistence in both the environment and the human body, PFAS, including PFOA, are often referred to as "forever chemicals." PFOA has been linked to a range of adverse health effects, including reproductive and developmental toxicity, hepatotoxicity, renal toxicity, immunotoxicity, neurotoxicity, genotoxicity, and carcinogenicity. However, the effects of PFOA on human vascular endothelial cells remain poorly understood, particularly in terms of sensitive time points where its impact may be most pronounced. The use of in vitro human cell models and new approach methodologies offer valuable alternatives to animal testing, providing relevant data for assessing the effects of PFOA on human health. In this study, we employed benchmark concentration modeling to derive and compare points of departure (POD) for transcriptomic changes (T-POD) in human vascular endothelial cells EA.hy926 following short-term (48 h, acute) and long-term (6 and 12 weeks, chronic) exposure to 1, 10, and 100 μ M and 1, 10, and 100 nM PFOA, respectively. Our mRNA sequencing results revealed a substantial number of differentially expressed genes (DEGs) in PFOA-exposed EA.hy926 cells across all concentrations and time points. The highest overall number of DEGs was observed after 6 weeks of exposure, with 10 nM PFOA yielding the highest total of 6,018 DEGs. The T-POD values for 6 and 12

weeks (4.1 nM and 22.1 nM, respectively) indicated greater sensitivity than the T-POD value derived from 48-h exposure (6.3 μ M). Functional gene analysis revealed that transcription was a sensitive, yet general molecular pathway affected by short-term exposure. After 6 weeks of exposure, the IL-17 signaling pathway was notably enriched, while after 12 weeks, pathways related to the extracellular matrix, cytokine-cytokine interactions, and cell adhesion were significantly affected. In conclusion, chronic exposure provided more informative data than acute exposure for assessing the risk of PFOA in human endothelial cells. Moreover, the transcriptomic data from long-term exposure offer valuable insights into early molecular pathways linked to PFOA exposure, highlighting their potential to connect toxicity effects across different biological levels.

1.11.P-Tu113 In Vitro Toxicological Assessment of PFAS Using Seahorse Extracellular Flux Analysis

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants with potential adverse effects on human and ecosystem health. This study, conducted as part of the European SCENARIOS project, investigates their toxicological effects on ZF4 zebrafish cells, with a focus on PFAS alternatives. The originality of our approach was to use Seahorse extracellular flux analysis to profile real-time changes in cellular metabolism. Through measurements of oxygen consumption rate (OCR) and proton efflux rate (PER), mitochondrial respiratory energy metabolism and glycolytic activity was assessed. PFAS-induced mitochondrial toxicity was also identified and quantified using the mitochondrial toxicity index (MTI). The traditional CCK8 assay was also employed to provide a comparative measurement of metabolic activity. To assess dose-dependent responses and metabolic disruptions, cells were exposed to a selection of legacy and alternative PFAS compounds, including legacy PFAS like PFOA, PFOS, PFDA and various perfluoroalkyl(poly)ether sulfonic acids at concentrations ranging from 10 to 1000 μ M for 24 hours. Additionally, a mixture containing PFBS, PFOS K salt, PF2EOESA, Nafion-BP2 and Nafion BP1 was tested to investigate potential additive, synergistic or antagonistic effects. Our results show significant alterations in OCR and PER, with compensatory shifts in cellular energy supply from mitochondrial ATP to glycolytic ATP, indicating PFAS-induced mitochondrial dysfunction. Nafion BP1, PFDA and PFOA were identified as the most toxic compounds, causing a strong inhibition of mitochondrial oxidative capacity as identified by the MTI. Interestingly, exposure to these compounds reduced cellular metabolic activity by nearly 50% at respective concentrations of 100 μ M, 300 μ M and 750 μ M. We confirm that perfluoroalkyl carboxylic acid toxicity increases with carbon chain length. The three most toxic compounds tested (7 - 10C) caused significant metabolic disruption, whereas shorter-chain PFAS (4C e.g., PFBA, PFBS and PF2EOESA) had minimal impact on ZF4 metabolism. These findings improve our understanding of PFAS toxicity at the cellular level, highlighting disruption mechanisms and providing key data to develop Adverse Outcome Pathways for PFAS, especially those with limited toxicity data. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 101037509 (SCENARIOS project).

1.11.P-Tu114 High Serum Concentrations of Perfluoroalkyl Substances (PFAS) in Norwegian Children and Associations with Adverse Health Effects

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The aim of this study was to assess PFAS serum concentrations in Norwegian children of different ages and to evaluate health outcomes associated with PFAS exposure. Several studies have examined PFAS concentrations in Norwegian children and their possible health effects. The results of the studies including 6 months old infants from Bergen, 12 years old children from Oslo and 15-19 years old teenagers from Tromsø will be presented. PFAS were measured by ultrahigh-pressure liquid chromatography tandem mass spectrometry. Observed detection rates for perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS) perfluorohexane sulfonate (PFHxS) and perfluorononanoate (PFNA) were 100%. Altogether 78% of the six-month-old infants from Bergen, 25% of the 12-year-old children from Oslo, and 87% of the teenagers from Tromsø had sum concentrations of these four PFAS (Σ4 PFAS) above the EFSA 2020 limit for maternal blood of 6.9 ng/mL. Serum concentrations of Perfluoroalkyl substances in Norwegian children (N = 1 153). Will be presented as a table.

1.11.P-Tu115 Comparative Acute Toxicity Assessment of Fluorinated and Fluorine-Free Firefighting Foams on Zebrafish Embryos

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The ubiquitous presence of per- and polyfluoroalkyl substances (PFAS) in the environment, particularly in groundwater near airports and former military training areas, presents significant challenges for research and remediation efforts. PFAS, synthetic chemicals prized for their water- and dirt-repellent properties, have been extensively used in various applications, including firefighting foams for class B fires in chemical industries and refineries. This study investigated the comparative acute toxicity of a fluorine-containing aqueous film-forming foam (AFFF) and an environmentally friendly, fluorine-free alternative (Vapurex), both manufactured by Dr. Sthamer. The fish embryo acute toxicity test was conducted using *Danio rerio* (zebrafish) embryos as the model organism. Embryos were exposed to various dilutions of both foam concentrates, ranging from 50% to 0.00000015%, based on the manufacturer's recommended 1% addition rate. Over a 96-hour exposure period, developmental abnormalities and mortality were observed and recorded. Acute mortality was evident for both foaming agents, particularly at higher concentrations (50%, 15%, and 1.5%). Notably, neither foam concentrate allowed for 100% survival across all tested dilutions. Dose-response curves were generated from the obtained results. Our findings indicate that both the fluorine-containing AFFF and the fluorine-free Vapurex exhibit clear acute toxic effects on *Danio rerio* embryos. However, to draw more definitive conclusions about the comparative toxicity of these firefighting foams to aquatic organisms, further studies exploring a wider range of dilutions and additional endpoints are necessary. This research contributes to the growing body of knowledge on the environmental impacts of firefighting foams and supports informed decision-making in the transition towards more environmentally friendly alternatives.

1.11.P-Tu116 Membrane–Water Partition Coefficients of Per- and Polyfluoroalkyl Substances (PFAS): A Promising Descriptor of their Bioaccumulation and Toxicity

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Per- and polyfluoroalkyl substances (PFAS) are chemicals of high environmental concern due to their widespread presence and high persistency in aquatic and terrestrial ecosystems. Yet, current PFAS exposure and risk assessment are inadequate, as they focus on only a limited subset of compounds, overlooking the large variation in chemical structures and properties of the more than 6000 CAS registered PFAS. Since it is not feasible to test the plethora of PFAS for their environmental fate and effects, a descriptor that is indicative of PFAS exposure, bioaccumulation and ecotoxicity is urgently needed. Classical descriptors like the octanol-water partition coefficient, have proven of limited value in evaluating the environmental behavior of PFAS, due to their unique characteristics and their affinity for phospholipids. Alternatively, the phospholipid membrane-water partition coefficient (K_{mw}), which determines the membrane permeability of a compound, could be used to assess the environmental fate, bioaccumulation and ecotoxicity of PFAS. Since data on the membrane-water partitioning of PFAS are scarce, the aim of this study was to measure the K_{mw} for a set of 17 PFAS with different structures, varying chain length and functional groups. To this end, two different methods were employed for the determination of K_{mw}. The first method was based on the evaluation of the retention capacity of the compounds on a chromatographic column coated with immobilized artificial membrane phospholipids (IAM-HPLC). The second method was based on the sorption of the compounds on solid supported lipid membranes (SSLM). Preliminary results of the first method (for 5 PFAS) aligned with K_{mw} values previously reported in literature. Both chain length and polar head played a role in the membrane affinity of the tested PFAS. The SSLM experiments are still ongoing and results were not available by the time of abstract submission. Overall, K_{mw} could be considered a useful descriptor of the bioaccumulation potential and toxicity of PFAS and consequently contribute to a more reliable environmental risk assessment of these forever chemicals.

1.11.P-Tu117 Applying Newly Suggested Simultaneous Analysis of Metabolomics and Lipidomics into Perfluorooctanesulfonate-derived Neurotoxicity Mechanism in Zebrafish Embryos

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To increase the reliability of data on changes in multi-layered biomolecules, a strategy for performing multi-omics with one sample is needed; however, related studies are lacking. A new strategy for the simultaneous analysis of metabolomics and lipidomics in zebrafish embryos was proposed and applied to explore the neurotoxicity mechanisms of perfluorooctanesulfonate (PFOS). Metabolite and lipid profiled simultaneously with methyl tert-butyl ether (MTBE) were compared with individual results from other extraction solvents. Behavioral alterations were measured after the zebrafish embryos were exposed to 0.1 20 μ M PFOS for 5 days. The metabolite-lipid profiles of the MTBE-based strategy analyzed with optimized larval pooling size of 30 were comparable to those of other extraction solvents, indicating the feasibility and efficiency of MTBE-based multi-omics analysis. Many metabolites and lipids, which were enriched more than those previously reported, completed the toxicity pathways involved in energy metabolism and sphingolipids, improving our understanding of PFOS-induced neurotoxicity mechanism manifested by increased movement under dark conditions. Our novel MTBE-based strategy enabled the multi-omics analysis of one sample with minimal use of zebrafish embryos, which will help advance multi-omics technologies in zebrafish toxicology for elucidating the toxicity mechanisms of per- and polyfluoroalkyl substances and toxic chemicals.

1.11.P-Tu118 Early Detected Snapshot of Neurodevelopment Toxicity in Zebrafish Embryos Exposed to Perfluorooctanesulfonate

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Rapid multi-omics approaches are essential for generating large-scale biomolecular data to build an inventory of per- and polyfluoroalkyl substances (PFAS) neurotoxicity; however, related studies remain limited. This study aimed to uncover biomolecular mechanisms of PFOS-induced neurotoxicity across developmental stages and identify early detection biomarkers in zebrafish embryos. Zebrafish embryos were exposed to 5 μ M PFOS from 4 hours post-fertilization (hpf) to 120 hpf, with 30 embryos with 6 replicates collected at 72, 96, and 120 hpf. Metabolites and lipids were simultaneously profiled using methyl tert-butyl ether (MTBE) extraction and analyzed with MetaboAnalyst 5.0. Although metabolite and lipid perturbations varied across developmental stages, consistent disruptions in energy metabolism and nervous system function were observed. Key biomarkers included Sulfatide 36:1 (d18:1/18:0), Sulfatide 38:1 (d18:1/20:0), 3,4-dihydroxyphenylalanine (L-DOPA), L-Citrulline, D-Tryptophan, acetyl-L-carnitine, eicosenoic acid, eicosadienoic acid, and arachidonic acid. The MTBE-based strategy efficiently captured dynamic metabolomic and lipidomic perturbation, identifying Sulfatide and L-DOPA as promising biomarkers of PFOS-induced neurotoxicity in developing zebrafish embryos. By enabling the rapid and simultaneous profiling of multi-layered biomolecules, this approach could be used to the early detection of PFAS-induced neurotoxicity and provide a robust framework for toxicity assessments of PFAS and other toxic substances in future studies.

1.11.P-Tu119 Immunotoxicity and Autotoxicity of Poly- and Perfluoroalkyl Substances in Fish

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Previous studies have reported the immunotoxicity of per- and polyfluoroalkyl substances (PFASs), but it remains a significant challenge to assess over 10,000 distinct PFASs registered in the distributed structure-searchable toxicity (DSSTox) database. In our study, we revealed differences in the immunotoxic responses of PFASs, which is dependent on the carbon chain length in zebrafish, providing new insights into the prediction and classification of PFASs mode of toxic action based on carbon chain length. In addition, given the potential degradation of α -perfluorooctane sulfonate precursor (PreFOS) into perfluorooctane sulfonic acid (PFOS) in vivo, there is still debate as to the specific nature of PreFOS-induced toxicity, whether autonomous or mediated by its metabolite PFOS. The findings from our study suggest that the immunotoxicity effects of α -perfluorooctane sulfonamide (PFOSA), an immediate PreFOS, are primarily due to its own toxicity rather than its metabolite PFOS. This conclusion was supported by dose dependent responses, the severity of observed effects, and multivariate analysis. In conclusion, our study reveals that the immunotoxicity of PFASs is related to carbon chain length and auto-toxic response, providing valuable insights for assessing the ecological risks of PFASs.

1.11.P-Tu120 Additive Effects of PFAS Mixtures on Acute Toxicity, Phenotypic and Behavioral Endpoints in Zebrafish Embryos

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Per- and polyfluoroalkyl substances (PFAS) are synthetic organic chemicals. Various adverse human health and environmental effects are associated with exposure to PFAS. Many studies described the effect of single PFAS compound in several model organisms, including the zebrafish embryo. However, fewer studies have considered PFAS mixture effects. To elucidate potential mixture effects of prominent PFAS we used a concentration addition (CA), a classical mixture modelling approach. We investigated the effects of single substances and mixtures containing PFOS, PFHxS, PFOA, PFNA, PFDA, and PFUnDA. One of the mixtures is based on acute toxicity effects (LCx) and was designed to exhibit equal toxicity according to CA modelling. Another is environmentally-relevant, based on mixture ratios found in the blood of Scandinavian populations where a concentration range of 0.5-179x of the human blood concentrations (HBC) were tested. Zebrafish were developmentally exposed using different exposure scenarios from 0-5 days post fertilization (dpf) to single substances or different mixture compositions. Acute toxicity, phenotypic, and behavioral effects were measured. At 5 dpf, zebrafish were subjected to an automated visual and acoustic motor response (VAMR) assay comprised of 26 behavior-based endpoints. Multiple behavior effects were observed at sublethal concentrations and included hyperactivity in dark-phase and acoustic startle response endpoints. This work identified a range of developmental and behavioral effects in zebrafish exposed to PFAS and indicate that the adverse effects of PFAS mixtures can be explained by compound additivity.

1.11.P-Tu121 Causal Analysis of PFAS Toxicity and Disease Associations Using Bayesian Networks with Data Integration

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Per- and polyfluoroalkyl substances (PFAS), a group of persistent environmental chemicals, have been associated with various health effects, yet the causal relationships with specific diseases remain complex and insufficiently understood. The aim of this study is to conduct a systematic review of the literature on PFAS toxicity and its associations with multiple diseases and to perform a causal analysis using Bayesian Network (BN) modeling. By integrating toxicity data, we seek to examine the strength and direction of PFAS-disease associations within a probabilistic framework. By consolidating findings from existing studies and incorporating quantitative data, we construct a BN to map connections between PFAS toxicity, potential biomarkers, and disease endpoints. The BN model identified potential causal pathways between PFAS and disease outcomes, including associations with endocrine functions. While some pathways showed stronger associations than others, the results indicate specific areas where PFAS exposure may influence disease risk, highlighting oxidative stress and inflammation biomarkers as key intermediate factors. This preliminary analysis underscores the utility of BN modeling for exploring PFAS-related health risks and identifying key pathways. Our findings provide an initial framework for prioritizing future research areas and refining causal models to better assess PFAS impacts on human health. This work was supported by Korea Environmental Industry & Technology Institute (KEITI) through 'Core Technology Development Project for Environmental Diseases Prevention and Management', funded by Korea Ministry of Environment (MOE) (2021003310005).

1.11.P-Tu122 Head To Head: How Polar Head Group Drives Toxicity Of Per- And Polyfluoroalkyl Substances To *Daphnia Magna*

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Per- and poly-fluoroalkyl substances (PFAS) are a ubiquitous group of anthropogenic compounds that have raised environmental concerns due to their persistent and bioaccumulative properties. To predict the ecotoxicity of the many untested PFAS, it is imperative to unravel the link between PFAS molecular properties and their ecotoxicity. So far, it has been established that PFAS ecotoxicity increases with increasing fluorinated carbon chain length, but still little is known about the influence of the polar head group on PFAS ecotoxicity. Therefore, the present study aimed to determine the ecotoxicity of PFAS with identical chain length, but different polar head groups to *Daphnia magna*. Four C4-PFAS were selected: perfluorobutanesulfonamide (FBSA), perfluorobutane sulfonic acid (PFBS), perfluorobutanesulfonyl chloride (PBSCI), and perfluoropentanoic acid (PFPeA). To compare their ecotoxicity, the effects of these four compounds on *D. magna* neonates (< 24h) mobility were tested according to OECD guideline 202. Six concentrations, including a negative control, were tested per PFAS, with four replicates per

concentration and five daphnids per individual replicate. Daphnid mobility was checked after 24 and 48 hours of exposure. Concentration-effect relationships were fitted to the experimental data from which EC50 values were calculated. The negative controls met the specified OECD requirements. Clear concentration-effect relationships were obtained for the effects of the four PFAS on daphnid mobility, with EC50 values of 12 mg/L FBSA, 1026 mg/L PFBS, 1961 mg/L PBSCl, and 2638 mg/L PFPeA. Hence, strong and significant differences in ecotoxicity between the four compounds were observed, driven by varying polar head groups. FBSA was the most toxic of the tested PFAS and was 220 times more toxic than the least toxic compound. The relatively high ecotoxicity of FBSA may be caused by a specific mode of action, as the sulfonamide head group may induce developmental toxicity. The low ecotoxicity of PFBS and PFPeA confirms their lack of specific modes of action. The sulfonate anion (R-SO₃⁻) of PFBS causes a higher affinity for sorption into the membrane, potentially causing the higher observed toxicity than its carboxylate homolog PFPeA and the non-dissociating PFAS PBSCl. It is concluded that the polar head group strongly influences PFAS ecotoxicity, emphasizing that PFAS environmental hazard strongly depends on their molecular properties. This research was supported by the Open Technology Programme of the Dutch Research Council (NWO) [grant number 18725]

1.11.P-Tu123 Discrepancies between CCK-8 and Trypan Blue Assays in PFAS-Exposed ZF4 Cells: Implications for Metabolic Stress Assessment

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Perfluoroalkyl substances (PFAS), particularly perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), are persistent environmental contaminants with potential toxicological effects. This study investigated the effects of PFOA and PFOS on zebrafish embryonic fibroblast (ZF4) cells, revealing critical methodological considerations for cytotoxicity assessments. ZF4 cells were exposed to various concentrations of PFOA and PFOS (100-1000 µM) for 24 hours. Cell viability was evaluated using the cell counting kit 8 (CCK-8) assay, trypan blue cell counting, and lactate dehydrogenase (LDH) assay, which measures cell membrane damage and LDH leakage from the cytoplasm. Surprisingly, the CCK-8 assay showed a dose-dependent increase in absorbance readings at concentrations up to 500 µM, particularly for PFOS, suggesting increased cell viability or proliferation. However, concurrent trypan blue cell counts revealed a dose-dependent decrease in cell numbers, with IC50 values determined for both PFOA and PFOS (570.1 µM and 586.1 µM, respectively). LDH assay results were consistent with trypan blue counts. This discrepancy between CCK-8 results and actual cell counts indicates that the CCK-8 assay may produce false-positive results in this context. The increased CCK-8 signal, despite reduced cell numbers, suggests that PFOA and PFOS exposure may induce metabolic stress in ZF4 cells, potentially leading to increased mitochondrial activity. These findings highlight the importance of using multiple assays to assess cell viability and stress responses, especially when studying PFAS toxicity in emerging model cell lines like ZF4. Furthermore, this research suggests the potential value of investigating mitochondrial bioenergetics in PFAS-exposed cells, as metabolic alterations may precede overt cytotoxicity. To further confirm enhanced metabolic stress, future work will include Seahorse experiments to measure cellular respiration and glycolysis. Additionally, mitochondrial functional studies will be conducted using flow cytometry to analyze MitoSOX and TMRM staining, providing insights into mitochondrial superoxide production and membrane potential, respectively. These results contribute to our understanding of PFAS toxicity mechanisms and provide critical methodological considerations for future toxicological studies, emphasizing the need for multiple assays in assessing cell viability and stress responses, particularly when studying emerging model cell lines. The authors gratefully acknowledge the Petroleum Technology Development Fund (PTDF) for awarding a Ph.D. scholarship to Timothy Prince Chidike Ezeorba (PTDF Scholar ID: 22PHD053). We also extend our appreciation to the Society of Environmental Toxicology and Chemistry (SETAC) Europe for providing a Student Registration Grant, enabling the presentation of our findings at the 2025 SETAC Europe Annual Meeting.

1.11.P-Tu124 Evaluation of Immunological Impact of PFAS Exposure on Cytokine Release and Gender-Based Responses in PBMCs

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Over the past few decades, humans have been constantly exposed to perfluoroalkyl substances (PFAS), a group of chemicals of major concern due to their widespread use in consumer goods and their resistance to biological degradation. The purpose of this study was to investigate the effects of PFAS on female and male peripheral blood mononuclear cells (PBMC), with a focus on immunomodulatory response by assessing both the pro-inflammatory and anti-inflammatory cytokines release following stimulation with mitogens. In our experiments, pools of human PBMCs from healthy female and male donors were plated

(3 x 10⁵ cells/well) and then exposed to 20 different PFAS congeners. The cells were initially exposed to three increasing concentrations of the chemicals, and then stimulated with either LPS (24 hours) or PHA (72 hours), separately. Stimulated cells, incubated in the absence of PFAS, were used as control. Supernatants were afterwards collected to assess the analysis. A protein microarray analysis of supernatants was performed to assess the release of 10 different pro-inflammatory and anti-inflammatory cytokines. Non-parametric pairwise multiple comparisons were used to test significance of differences in cytokine levels between treated and control cells. Our preliminary results indicated a statistically significant reduction in cytokine concentrations in mitogen-stimulated PBMCs exposed to PFAS. Notably, we observed a more pronounced alteration in the cytokine profile of PBMCs from female donors treated with PFCA group, compared to those from male donors, suggesting a potential gender-specific effect. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101037509 (SCENARIOS project).

1.11.P-Tu125 Toxicokinetics of PFOS in Freshwater Macroinvertebrates

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The Increasing use of PFOS has led to their release into the aquatic environment. Toxicokinetics is a key component in the assessment of a chemicals risk to the environment and human health, and is a requirement of many global regulations for chemical management. Chemicals that bioaccumulate are of concern because they have the potential to achieve high concentrations in biota, which can lead to toxicological effects. However, there is only limited knowledge about the effects of long-term exposure to PFOS on freshwater macroinvertebrates. To fill this knowledge gap and to improve our mechanistic understanding of the effects of PFOS on freshwater macroinvertebrates, the current study explored the toxicokinetics of PFOS in two insects and one crustacean species. The tested species in these experiment were the mayfly *Cloeon dipterum*, the phantom midge *Chaoborus obscuripes* and the freshwater shrimp *Gammarus pulex*. In the toxicokinetics test, organisms were exposed to 100 µg/L of PFOS for 3 days during the uptake phase and then transferred to clean water for 3 days of elimination. The selection of the exposure concentration during the uptake phase was based on our previous chronic toxicity results for the same species. The survival for the three species at the end of experiment was 77, 63 and 88% for *C. dipterum*, *C. obscuripes* and *G. pulex*, respectively. All water samples have been measured by LC-MS/MS, and the exposure concentrations were within 90 to 130% of the nominated concentration, with an acceptable deviation. During the elimination phase, about 0.05 to 0.7 µg/L of PFOS was detected in the water. *G. pulex* eliminated the highest amount (0.7 µg/L) compared to *C. dipterum* and *C. obscuripes* which eliminated around 0.2 µg/L. These results are quite interesting and suggest that *G. pulex* may eliminate more PFOS than the other two species. Furthermore, the internal concentration measurements is in progress and will be presented together with some preliminary TK modelling results. PUNJAB EDUCATION ENDOWEMENT FUND PUNJAB, PAKISTAN.

1.11.P-Tu126 An In-depth Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Prenatal and Cord Blood Samples

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PFAS are pervasive environmental pollutants found in air, water, food, and consumer products. Their chemical properties, such as persistence, bioaccumulation, and resistance to degradation, make them of concern for human and ecological health. Common PFAS compounds, including PFOS and PFOA, have been detected in human serum with half-lives of several years. Global trends show declining PFAS levels in some regions following regulatory phase-outs, but stable background levels persist in low-exposure areas. This study examines PFAS levels in maternal and cord blood samples, exploring their transfer from mother to foetus and assessing the presence of branched and linear isomers. Samples were collected at 38 weeks of pregnancy and at birth, processed with a generic extraction method, and analysed via high-resolution mass spectrometry for 25 target PFAS, 38 suspect and over 6000 library associated fluorinated compounds. The analysis identified PFOS, PFDoA, PFUdA, PFDA, and PFOA as the most frequently detected compounds. Some emerging PFAS compounds were found in limited samples, while short-chain PFAS, such as PFBA and PFBS, were detected infrequently, reflecting their rapid excretion and localized exposure. Statistical analysis revealed significant differences in PFAS concentrations between maternal and cord blood, with some compounds higher in one matrix than the other. Suspect screening revealed over 200 potential PFAS features, though many represent noise or low-confidence identifications. Variations in detection frequencies and concentrations align with trends reported in other human biomonitoring studies. Notably, branched isomers of PFOS accounted for up to 30% of the detected PFOS, demonstrating diverse exposure sources and potential health implications. This work highlights the challenges of PFAS detection, including the influence of laboratory blanks, instrumental noise, and the diversity of isomers. It emphasizes the need for robust analytical methods to better understand PFAS

exposure and their impacts on maternal and foetal health. Samples were supplied and extracted at RECETOX, Masaryk University, while all data processing and investigation was done at Exeter University UK.

1.12.A Endocrine Disruption Assessment: Opportunities to Enhance and Complement Current Approaches

1.12.A.T-01 Animal-free In Vitro Assessment of Receptor-Mediated Endocrine Activity Including Phase-1 Metabolism

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Substances affecting the endocrine system are present in the environment in complex mixtures and pose a risk to organisms and entire ecosystems even at low concentrations, which increases the importance of considering and evaluating endocrine disruptors. For this, standardized in vitro methods according to OECD test guidelines 455 and 458 can be applied to determine the agonistic and antagonistic effect on estrogen and androgen receptors on a cellular level. However, these methods have disadvantages of (1) containing undefined animal components like Fetal Bovine Serum (FBS) possibly leading to less reproducible results and ethical concerns and (2) a lack of metabolic representation of whole organisms which could lead to an over- or underestimation of the samples endocrine activity. In this study, the methods according to OECD TG 455 and 458 should be improved by adapting two of the OECD-relevant cell lines (ER- α -CALUX[®] and AR-CALUX[®]) to FBS-free conditions and the supplementation with external biotransformation systems (S9 homogenates) derived from rats/humans or their respective animal-free alternative to evaluate the endocrine activity of model substances after metabolism. Overall, the effects without/with metabolism and without/with adapted cell lines will then be compared to assess the assay adaptation and their (beneficial) impact on risk assessment. In the first step, both cell lines could be successfully adapted to a self-developed medium with few ingredients. The sensitivity of the cells to the reference substances 17 β -estradiol (ER- α -CALUX[®]) and dihydrotestosterone (AR-CALUX[®]) was comparable under FBS-containing and FBS-free conditions and similar concentration-dependent response curves could be generated. In the second step, a set of different model substances will be tested for their (anti-)estrogenic or (anti-)androgenic effect with and without metabolism, which will allow an initial comparison of the metabolic performance of the different S9 mixes in endocrine testing. Overall, the comparison of the endocrine effect of several model substances in FBS-containing versus FBS-free medium with and without the addition of S9-homogenates will enable a more comprehensive evaluation of the new method and thus possibly demonstrate that in vitro tests can also be carried out reliably without the use of animal components.

1.12.A.T-02 In Vitro Potencies of Chemicals for Inhibition of Thyroperoxidase (TPO) in Four Fish Species Compared to Mammalian Potencies in the ToxCast Database

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There are many concerns about chemicals in aquatic environments that can disrupt thyroid hormone signaling (THS) in fish. THS is a conserved process, regulated by a series of coordinated events involving several proteins. Additionally, THS in fishes is vital to many biological functions such as growth, development, and metamorphosis. Thyroperoxidase (TPO) is the main enzyme involved in synthesis of the thyroid hormones, tetraiodothyronine (T4), which is converted to the biologically more active form: triiodothyronine (T3). Chemical additives, pharmaceuticals, pesticides, and personal care products in the environment can inhibit the actions of TPO, leading to changes in THS and an overall decreased synthesis of T3 and T4, and a possible decrease in systemic concentrations of T3 and T4. Currently, screening for chemicals for TPO inhibiting properties has focused on mammalian-based assays, such as those used in the United States Environmental Protection Agency's (USEPA) ToxCast Program. In fish, the limited chemical safety investigations have focused almost entirely on a single non-native model species, the zebrafish (*Danio rerio*), which may not be representative of all fish species for THS disruption. Thus, little is known about whether chemicals with TPO inhibiting properties in mammals share these properties in fishes and whether there are differences in sensitivities among different species of fish. The present study investigates inhibition of TPO enzymes, using high-throughput animal alternative methods by adapting the previously developed in vitro Amplex Ultrared-Thyroperoxidase (AUR-TPO) assay for use in fishes. Through this, species sensitivity to TPO inhibition between fish and mammals to xenobiotics can be

compared. Chemicals with a range of potencies were chosen from the US EPA CompTox Database. Results of this study will determine whether fish species differ in their sensitivity to TPO inhibitors and how potencies compare to mammalian-based assays. Funding was provided by Louisiana Board of Regents

1.12.A.T-03 Metamorphosis as an Endpoint for Thyroid Hormone Disruption in Fish

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Metamorphosis is a vital biological process characterized by the transition from larval to juvenile stage of development and is most commonly associated with amphibians. Pesticides, chemical additives, pharmaceuticals, and personal care products found in surface waters can disrupt thyroid hormone (TH) signaling leading to accelerated, delayed, or prevented metamorphosis. TH disruption has been well studied in mammals and amphibians, and amphibians are generally sensitive to metamorphic impacts from a wide range of chemicals. In fishes, TH disruption studies have focused on small model species with simple metamorphosis, such as zebrafish (*Danio rerio*), which are relatively insensitive to disruption. However, some fish species complete a metamorphosis as complex as amphibians and it is unknown whether these fishes with dramatic transformations are more sensitive to chemical disruption relative to zebrafish. The present study aimed to (1) develop and apply an in vitro assay to screen thyroid hormone receptor (THR) disruption in fishes with varying degrees of metamorphic complexity using a known TH agonist, triiodothyronine (T3) and (2) develop a fish metamorphosis toxicity assay using spotted gar (*Lepisosteus oculatus*) to assess sensitivity to metamorphic chemical disruption in vivo for species with dramatic transformation. The in vitro assay utilizes COS-7 cells transfected with THR constructs for the species of interest, a thyroid response element vector, a reporter vector containing a luciferase gene, and a pRL-TK internal control vector. Cells were exposed to T3 at serial concentrations using 96-well plates, incubated in the dark, and read on a luminescence plate reader following the addition of a dual-luciferase agent. Exposures for gulf killifish (*Fundulus grandis*), spotted gar, southern flounder (*Paralichthys lethostigma*), and American eel (*Anguilla rostrata*) showed median effective concentration (EC50) values ranged by just 8-fold, from 0.0001nM to 0.0008nM. This suggests that sensitivity to THR disruption might not be driven by metamorphic complexity, at least not for T3. There is no current knowledge on whether a lack of species sensitivity differences translates to a lack of differences in metamorphosis. To address this, the in vivo assay will evaluate susceptibility to metamorphic disruption by exposing embryos to sub-lethal concentration of T3 during metamorphosis and comparing exposed individuals to previously identified metamorphic milestones.

1.12.A.T-04 Immunotoxic Effects of Endocrine Disruptors – An Understudied Adverse Outcome in Fish

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Endocrine disrupting chemicals (EDCs) are among the most studied environmental pollutants in (eco)toxicology. Despite their scientific and regulatory recognition, little attention has been given to their immunotoxic effects, and current research and data on this topic is mainly limited to human health impacts. Regarding the environment, there is still a lack of knowledge and test protocols for immune-related outcomes in fish. While the impact of estrogenic EDCs on the fish immune system has raised some attention in the last decade, thyroid hormone disrupting compounds (THSDCs) and their effects on the fish immune system are less well studied. This presentation is aimed at describing the immunomodulatory roles of environmental pollutants by summarizing the existing research on the immunotoxicity of EDCs in fish, with focus on the TH system. A literature review was performed on studies about THSDCs and their role in disruption of the fish immune system at different levels of biological organization. The results were summarized according to the adverse outcome pathway (AOP) concept to create a comprehensive overview and visual representation of the results. Additionally, two examples of ongoing research about immunotoxic effects on fish are presented to show which methods can be used in a regulatory context to assess immunotoxicity in fish. As a short-term new approach methodology (NAM), we developed a protocol using zebrafish embryos infected with fluorescently labeled mycobacteria and exposed to potential immunotoxicants. In the context of the ZeroPM project, the effects of 11 PFAS on the innate immune function of zebrafish embryos was assessed. Two of the PFAS caused increased bacterial loads compared to control embryos, which confirms their immunotoxicity described in humans. In the SUSPECT project, a long-term experiment with rainbow trout exposed to sodium fluoride (NaF) and

infected with a virus, THSD and immunomodulatory effects were observed at different levels of organization (health status, survival, morphometry, behavior, histopathology, RNAseq). Overall, this work demonstrates that THSDCs can alter fish immune system functioning along the AOP and therefore lead to reduced survival after pathogen infection. Our zebrafish immunotoxicity assay has the potential to be used for the assessment of immunotoxic effects in fish that could be implemented in the OECD testing framework for environmental health and potential extrapolation to humans. Funding was provided from: European Union's Horizon 2020 Research and Innovation Programme under grant agreement 101036756 (ZeroPM). National Environment-Health-Work Research Program of Anses with the support of the Ministries of Environment, Agriculture and Labor (ANSES-22-EST-050) (SUSPECT).

1.12.A.T-05 Elucidating the Mode of Action of Priority Pollutants on the Thyroid Axis by Combining Two Eleuthero-embryonic Bioassays

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Each endocrine axis (e.g., thyroid, estrogenic) can be impacted by chemicals acting through various modes of action (MoA), including receptor interaction, hormone production, transport, or elimination. We propose a method for elucidating the MoA of thyroid disrupting chemicals while addressing two main problems: I) lack of assays for thyroid-active chemicals that utilize fish, II) ethical concerns regarding in vivo testing. One of the OECD test guidelines for identifying endocrine activity is the XETA test (Xenopus Eleuthero-embryonic Thyroid Assay). This assay uses a transgenic *Xenopus laevis* line harboring a gene coding for GFP under the control of a promoter responsive to thyroid signaling. Fluorescence changes in embryos indicate thyroid axis activity. Additionally, the XETA assay provides insights into the MoA by comparing fluorescence responses in the absence and presence of thyroid hormones. However, some MoA are not detected by XETA. To address this gap, while also providing a fish-based assay for thyroid active chemicals, a new test, TETHYS (Transgenic Eleuthero-embryo Thyroid Specific test), was developed based on a transgenic medaka fish line. In this assay, GFP expression is driven by the thyroglobulin promoter, marking thyroid follicles. Notably, the TETHYS assay can detect MoA like iodine import inhibition, which the XETA cannot. These two tests have two major advantages: they are short (72h) and they are based on the use of eleuthero-embryos which are not regulated under the European Directive on animal experimentation. The goal is to combine the XETA and the TETHYS assays to develop a comprehensive method for elucidating thyroid-disrupting MoA that benefits from whole organism testing, short duration of the tests, and a more ethical approach. This combined approach was already applied to reference substances with known MoA. Following this, 16 priority substances proposed by ANSES will be assessed in the same way. For those chemicals that are showing thyroid axis activity, we will be able to propose possible MoA by comparing the results with those generated for the reference chemicals. Furthermore, transcriptomics analysis will be performed for two of these thyroid disrupting chemicals. This will further help with a more precise identification of their MoA. The work presented here was partly funded by ANSES under grant number ANSES-23-EST-098, and the INCLUE HORIZON MSCA-DN project.

1.12.B Endocrine Disruption Assessment: Opportunities to Enhance and Complement Current Approaches

1.12.B.T-01 Tiered Assessment Scheme Linking NAMs to Adverse Outcomes to Identify Thyroid Disruptors in Aquatic Vertebrates

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According to the World Health Organisation and European Commission definitions, substances shall be considered as endocrine disruptors if they show adverse effects, have endocrine activity and the adverse effects are a consequence of the endocrine mode-of-action (using a weight-of-evidence approach based on biological plausibility), unless the adverse effects are not relevant to humans or non-target organisms at the population level. To date, there is no decision logic on how to establish endocrine disruption via the thyroid modality in non-mammalian vertebrates. We present an evidence-based decision logic to identify thyroid-mediated effect patterns in aquatic vertebrates, i.e. currently amphibians. The decision logic

includes internationally agreed test guidelines and proposes detailed considerations on how to select relevant assays and interpret the findings. It combines New Approach Methodologies with higher-tiered tests up to refined assessment including population modelling. If the mammalian dataset, including any in vitro data, is used as the starting point and indicates no thyroid-mediated adversity or mode-of-action, the *Xenopus* Eleutheroembryonic Thyroid Assay (XETA) may be conducted in place of the Amphibian Metamorphosis Assay (AMA) or its extended, fixed termination stage variant (EAMA) to exclude thyroid-mediated activity in non-mammalian vertebrates. The AMA or EAMA may be performed if there are indications for thyroid adversity in the mammalian dataset or following a positive XETA. The AMA and EAMA inform on both thyroid-mediated activity and potentially population-relevant adversity and consider generalized toxicity occurrence. In evaluating findings, the response patterns of all assay endpoints are considered, including both the consistency and direction of changes. Thyroid-mediated effect patterns identified at the individual level in the amphibian tests are followed by a mode-of-action analysis and where necessary a population relevance assessment. Finally, all data are considered in an overarching weight-of-evidence evaluation. The decision logic can be adapted, e.g., to accommodate fish tests once thyroid endpoints are included therein. It ensures that all scientifically relevant information are considered and that animal testing is minimised, and it can be included in regulatory assessments to facilitate the conclusion on whether substances meet the endocrine disruptor definition for the thyroid modality in non-mammalian vertebrates.

1.12.B.T-02 Assessing the Impact of Dietary Restriction on Endpoints in the Fish Short-Term Reproduction Assay: Implications for Interpreting Endocrine Activity

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The Fish Short-term Reproduction Assay (FSTRA) is an important in vivo endocrine screening tool used to detect potential endocrine activity of chemicals on the hypothalamic-pituitary-gonadal (HPG) axis. This study aimed to investigate whether a non-chemical stressor, dietary restriction, could influence FSTRA outcomes, particularly in relation to endpoints typically linked to HPG axis activity, as well as effects on fecundity, fertility, and GSI. A standard Test Guideline compliant FSTRA was conducted using five different feeding regimes, providing 100%, 30%, 15%, 7.5%, and 2.5% of the standard laboratory feeding ration. All endpoints outlined in the TGs, including gonad histopathology, were assessed, and the specificity of these endpoints in determining EAS-mediated activity was evaluated. Statistically significant reductions in fecundity, wet body weight change (both sexes) and male GSI were observed across all reduced feed rations (30% to 2.5%), following a clear feed-dependent response. Female GSI was significantly reduced at the 2.5% ration, although non-significant reductions were seen across all rations compared to the reference (100%) ration. A clear monotonic decline in female VTG levels occurred, with statistically significant decreases in the 7.5% and 2.5% ration groups when compared to the reference ration. No statistically significant effects on VTG were observed in males. There were no statistically significant changes in male tubercle score or reductions in fertilization success. Histopathology investigations are on-going, and all results will be available at the time of the conference. These results highlight the need for appropriate Maximum Test Concentration setting that does not focus solely on mortality (e.g., acute LC50 testing), since sub-lethal effects can clearly influence responses in FSTRA studies and these should not be mistaken for evidence of specific endocrine activity. Further, holistic interpretation of all endpoints should be employed to avoid unnecessary repeat or higher tier studies to clarify such non-specific responses. Such approaches would provide a more comprehensive basis for concluding on the endocrine activity of a test substance, and help to differentiate endocrine-mediated effects from non-specific toxicities.

1.12.B.T-03 The Population Relevance of Effect in Mammals: Experience and Future Perspective

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Generally, for environmental risk assessment the entity to be protected in reproductive risk assessment is the population. The selection of the relevant ecotoxicological endpoint as well as the assessment for endocrine disruption in wild mammals are both based on the use of toxicity mammalian toxicology studies. Considering the level of protection for wild mammals (population) is different than the one for human health (individual), effects in mammalian species often need to be reconsidered from a different perspective. When assessing the effects observed in the available toxicological studies, relevant parameters for the effects on wildlife populations are those parameters that show an expectation of adverse effects on the population in the field. Effects on growth (body weight and length), development and reproduction (such as fecundity, fertility, sex ratio, hatching success and offspring survival) in single species in laboratory studies are generally regarded relevant for the maintenance of the wild populations.

Regarding the effect level, a statistically significant difference compared to the control in a relevant parameter and the biological relevance of the observed change should be considered to assess if an observed change is adverse effect at the population level. A number of substances evaluated so far by EFSA have been re-analyzed to report examples of how the assessment for population relevance of adverse effects in mammals has been performed both in the context of endocrine disruption and for the selection of the ecotoxicological relevant endpoint. Based on experience, drawing a conclusion on population relevance is more challenging when effects at organ level are observed. For example, in the context of assessments for endocrine disruption, it may be controversial to conclude on the relevance of effects like increase in the incidence and severity of follicular cell hypertrophy. In future, it would be beneficial to come to an agreed list of apical effects that may be related to a thyroidal mode of action in mammals. In addition, useful tools for better understanding the population relevance of effects observed in laboratory toxicity studies, e.g. effect models, are currently under discussion for their development and possible implementation.

1.12.B.T-04 Risk Management of Environmental Endocrine Disrupting Chemicals (EDCs) - Derivation of Predicted No Effect Concentrations (PNECs)

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According to Article 60 of the UK Registration, Evaluation, Authorisation and restriction of Chemicals (REACH) legislation, authorisation of a Substance of Very High Concern (SVHC) can be granted in one of two ways: either where adequate control has been demonstrated (with exposure concentrations below an acceptable threshold) or due to socioeconomic need. To date, authorisations for SVHCs that are environmental endocrine disrupting chemicals (EDCs) have only been granted on socioeconomic grounds. There is however, no guidance to applicants about the circumstances under which an acceptable concentration might be determined. A SETAC Pellston® workshop on Ecotoxicological Hazard and Risk Assessment Approaches for Endocrine-Active Substances held in 2016 concluded that a predicted no effect concentration (PNEC) can be determined for EDCs provided that sufficient data are available. We wanted to explore what this means in practice. Our project reviewed three data rich EDCs to establish what PNECs might be considered sufficiently protective of endocrine effects in the aquatic environment, and then applied the findings to eight further EDCs with fewer data to test the concept. Ecotoxicity datasets were compiled and their reliability was assessed before they were used to determine relevant PNECs for each substance. The approach covered oestrogen, androgen, and steroidogenesis (EAS) modalities due to insufficient data being available for thyroid-mediated effects. The report suggests that it is possible to derive precautionary threshold values for EDCs based on no-observed effect concentrations (NOECs) using an appropriate assessment factor (AF). Conventional AFs are considered acceptable where Organisation for Economic Co-operation and Development (OECD) Conceptual Framework (CF) Level 5 data are available, because such studies are multi-generational and cover relevant life cycle traits. However, they are not sufficiently protective of endocrine effects where only OECD CF Level 3 and 4 data are available. Our proposal is to increase the AF in such circumstances, to reflect the higher uncertainty. For one substance which only had an OECD CF Level 3 fish short term reproduction assay (FSTRA) available, our approach was able to derive a PNEC that would be protective for the lowest biomarker NOEC concentration. It is also important that other chronic ecotoxicity data are considered: for two pesticidal substances, algae were more sensitive than fish.

1.12.B.T-05 An Integrative Framework for the Identification and Prioritization of the Risk Assessment of Endocrine Disrupting Chemicals (EDCs)

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Endocrine-disrupting chemicals (EDCs) affect human health and ecosystems, largely contributing to the current increase in chronic diseases and to worldwide biodiversity loss. For humans, EDC exposure induces metabolic and neurodevelopmental disorders and is widely associated with the reduction of fertility rates in developing countries. For the environment, chemical pollution interferes with ecosystem homeostasis and disrupts the food chain, leading to the irreversible shrinking of bird, amphibians and fish populations across the globe. Despite EDCs being a priority for regulatory agencies, only a small fraction of chemicals authorized for commercial use in the European market have been characterized for ED-activity. To streamline this process, *in silico* methods are becoming fast and reliable tools to infer chemical toxicity. The aim of this project is to generate and validate a machine learning framework to identify and rank chemicals according to their potential ED-activity. To do so, we first (1) generated an integrative and multidisciplinary database containing physicochemical, human toxicological, and

ecotoxicological properties of more than 100 000 EU-relevant chemicals. The database includes over 80 000 parameters, including partition coefficients, chemical-gene/protein associations, hormone binding assays, ecotoxicity for aquatic species and similarity to structural alerts related to endocrine disruption, among others. Secondly, (2) we developed a web-based exploratory tool that allows users to explore chemicals, their properties, and structural similarities. Thirdly, (3) we implemented a machine learning model to identify and rank chemicals based on their potential ED-activity. To improve the transparency of the model, SHapley Additive exPlanations (SHAP) was used to define chemical properties contributing the most to each prediction. Finally, (4) the top-ranking unknown EDCs will be further validated using in vitro reporter assays. In conclusion, we are developing a unique, integrative and multidisciplinary open-source resource for scientists, regulators and industry to evaluate and identify novel EDCs. Our outcomes may be used to prioritize the risk assessment of EDCs, thereby contributing to promote a shift in EU chemical regulations towards a toxic-free environment. The project is funded by the Brussels-Wallonia Federation.

1.12.P Endocrine Disruption Assessment: Opportunities to Enhance and Complement Current Approaches

1.12.P-Tu127 Learnings from Identification of Endocrine Disrupting Properties for the Environment under REACH with focus on Mode of Action Analysis

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So far, 17 substances have been identified and included in the Candidate List of Substances of Very High Concern (SVHC) due to their endocrine disrupting (ED) properties for the environment. A substance is considered to have ED properties for the environment if a biologically plausible link can be established between an altered function of the endocrine system and an adverse effect relevant at the population level for wildlife. The major challenge in identifying substances as having ED properties for the environment is to establish a biologically plausible link between the observed endocrine activity and adversity. This is particularly challenging for the environment because the number of endpoints in the available guideline studies is limited. The study aims to collect learnings from the previous assessments to facilitate future regulatory assessments of ED properties for the environment. The ultimate aim is at providing advice on how to improve existing Adverse Outcome Pathways (AOPs) and regulatory test guidelines to better facilitate identification of ED properties. As a first step, an analysis was conducted on the previously identified substances focusing on the mode of action (MoA). Thereafter, for each substance, the type of data used to support the molecular initiating events (MIE), intermediate key events (KEs), and adverse outcomes (AOs) has been analysed in detail i.e. guideline versus non-guideline studies from open literature or databases. A database has been created which maps the postulated MoAs to existing AOPs. This allows evaluation of patterns across substances and assessment of areas needing further investigation. Preliminary findings reveal recurring MoAs across several substances. In particular, the most frequent MoAs could be assigned to the following AOPs in the AOPwiki: AOP 29 Estrogen receptor agonism leading to reproductive dysfunction and AOP 52 ER-agonism leading to skewed sex-ratio due to altered sexual differentiation in males. Both of these AOPs concern estrogenic MoA, however neither of them are endorsed by OECD and would merit prioritisation for inclusion in the OECD work program. In addition, we identified other KEs that warrant further investigation and potential inclusion in the next AOP developments. This study supports future assessment of ED properties for the environment by identifying the most common MoAs and provides recommendations for future AOP development related to environmental endocrine disruption.

1.12.P-Tu128 Use Of The Weight-of-evidence In The Identification Of Endocrine Disruptors Under REACH

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Weight-of-evidence (WoE) approaches have been used for many years with the ultimate goal to synthesize complex information for robust and transparent decision-making. The term WoE is neither a scientifically well-defined term nor an agreed formalised concept, therefore, methods and approaches may vary. A WoE approach is for example used as the basis of the endocrine disruptor (ED) assessment at the EU level, where EDs can be identified as substances of very high concern (SVHC) under the REACH regulation and classified in new hazard classes under the CLP regulation. For the identification of ED, the WoE approach consists of gathering, assembling, weighing and integrating information to determine the extent of support for ED properties, i.e. whether there is the presence of endocrine activity, adverse effect(s) and a biological plausible link between the adverse effect(s) and the endocrine activity. In the commission

delegated Regulation (EU) 2023/707 of December 2022 amending the CLP Regulation (EC) No 1272/2008, several important factors are listed to be considered when assessing ED using a WoE approach: (a) positive and negative results; (b) the relevance of the study design for the assessment of adverse effects (including at the population level) and endocrine activity; (c) the adverse effects on reproduction, growth/development, and other relevant adverse effects which are likely to impact on populations; (d) the quality and consistency of the data; (e) the route of exposure, toxicokinetic and metabolism studies; (f) the concept of the limit dose (concentration), and international guidelines on maximum recommended doses (concentrations) and for assessing confounding effects of excessive toxicity; (g) where available, adequate, reliable and representative field or monitoring data or results from population models. While no chemical has yet been classified under CLP for ED properties to the environment, our work aims at assessing how the different factors mentioned above have been considered in the WoE assessment of ED for the environment under REACH. Using a database of about 20 substances, we will focus on aspects such as reliability and relevance, and how the lines of evidence for assessing the biologically plausible link between endocrine activity and adversity are structured. This work will help identify potential areas of improvement for future identification of endocrine disruptors under the CLP regulation. The views expressed in this abstract are solely those of the authors and the content of the poster presentation does not represent the views or position of the European Chemicals Agency.

1.12.P-Tu129 Developing the new CLP Guidance on Endocrine Disrupting properties: Stakeholder engagement, key considerations and discussion points

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The Commission Delegated Regulation (EU) 2023/707 of December 2022 has amended the CLP Regulation (EC) No 1272/2008 by adding new hazard classes and criteria for the classification, labelling and packaging of substances and mixtures. This regulatory action was prompted by concerns that substances with endocrine disrupting (ED) properties were not covered by existing hazard classes. The classification of a substance as ED is intended to indicate that the substance may cause an adverse effect via an endocrine mode of action. The ED classification refers separately to EDs for human health and for the environment. The ED criteria require evidence on three elements: adverse effect(s), endocrine activity, and a biological plausible link between them. Ultimately, the ED environment classification will result in allocation of substances to one of two categories, Category 1: Known or presumed endocrine disruptors for the environment or Category 2: Suspected endocrine disruptors for the environment. The new hazard classes have led to a need to develop guidance on the application of the CLP criteria for these hazard classes. The European Chemicals Agency has been managing the process of harmonised classification and labelling for substances and mixtures since the adoption of CLP in 2008 and led the work on the development of the CLP guidance for the new hazard classes. All relevant ECHA stakeholders, namely Member States, the European Commission, EFSA, industry and NGOs participated in the development of this guidance document. The development of the guidance took place from early 2023 until the publication of the guidance in November 2024. This poster will present the process of development of the guidance document and will illustrate the key considerations for reaching a conclusion on the classification. In particular, it will describe the necessary steps to perform a weight-of-evidence assessment, namely the collection of all available relevant information, its evaluation and the decision logic for the classification of substances and mixtures as endocrine disrupting substances as well as the differences of Category 1 and Category 2 classification. It will also discuss the issues that were more controversial during the guidance development for the ED environment such as the approaches regarding non-EATS modalities and population relevance.

1.12.P-Tu130 Variability of Vitellogenin in Fish Studies for Endocrine Assessment

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Vitellogenin (VTG) is an in vivo mechanistic parameter relevant for the assessment of endocrine properties of chemicals impacting the Hypothalamic Pituitary - Gonadal (HPG) axis in non-target organisms. A recurrent issue with VTG observed in the fish studies submitted for ED assessment of PPPs is a high degree of variability, potentially leading to false negatives. Such variability could be intrinsically related to the fish (e.g., species, age) but also other external variables may have a significant contribution. The impact of the variables on this issue is currently unclear. Therefore, the aim of this work is to understand the impact of different internal and external variables on the variability of VTG, which will set the basis to i) fully trust the available data in the context of the ED assessment and to ii) provide useful indications to laboratories performing the experiments in order to harmonize the procedures leading to the

measurement of VTG. A data extraction exercise was carried out on the fish studies submitted for the ED assessment in the context of PPP approval/renewal. VTG data were extracted from individual fish from negative and solvent controls. Physiological parameters of the fish (e.g., age, sex, breeding), external experimental factors (e.g., performing laboratory, experimental conditions, test design, solvent used), and available information for preparation and analysis of VTG were collected. Preliminary results indicate high variability in the VTG measurements. Differences were observed across different laboratories, with two laboratories indicating better consistency in the VTG measurements, potentially related to their level of experience. However, none of the investigated variables e.g., age, testing conditions, demonstrated a significant contribution to the observed variability. We hypothesize that different laboratory practices, which are generally not extensively reported in the study reports and could not be captured in our analysis, may explain the observed differences across laboratories. A relationship with the fish stocks maintained by the different laboratories prior the analysis cannot also be excluded. In conclusion, our work supports the need for harmonization in the VTG analyses across different fish studies in order to fully trust the available data in the context of the ED assessment and avoid useless sacrifice of vertebrate animals. This work is presented under the sole responsibility of the authors who are currently employed with the European Food Safety Authority (EFSA), and may not be considered as an EFSA scientific output. The positions and opinions (if any) presented in this article are those of the authors, and do not necessarily represent the views of EFSA.

1.12.P-Tu131 Amphibian Studies Investigating the Endocrine Disrupting Properties of Chemicals: A Comparison of their Statistical Power

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Amphibians are the current model species for investigating the endocrine properties through the Thyroidal modality in non-mammalian species. Two standardized in vivo amphibian study designs are available to sufficiently investigate the endocrine activity and/or adversity of plant protection products (PPPs) through the T-modality. The Amphibian Metamorphosis Assay (AMA, OECD 231) is a mechanistic screening assay, where *Xenopus laevis* tadpoles are exposed since stage Nieuwkoop & Faber (NF) 51 for 21 days. The Larval Amphibian Growth and Developmental Assay (LAGDA, OECD 241) instead entails exposing *X. laevis* embryos at NF stage 8-10 to a minimum of 4 different concentrations of the test chemical as well as control(s) until 10 weeks after the median time to reach metamorphosis (identified as NF stage 62, hereafter time to reach NF62). The LAGDA is the recommended test to sufficiently investigate the endocrine adversity for the T-modality, since it gives primary emphasis to potential population relevant effects. A burning question in the regulatory endocrine assessment of pesticides is whether conducting a LAGDA is a necessary step to address concerns identified by a positive AMA. Another one is the consideration of the non-standard fixed-stage version of the AMA, which extends until tadpoles reach stage NF62, aligning with the measurement of the time to reach NF62 carried out in the LAGDA. To further clarify some of the uncertainties around the use of LAGDA, and to help further consideration of the Extended AMA (EAMA) in regulatory context, the statistical power of the three test designs was tested for all the parameters entailed to be measured in the respective study designs (except for thyroid histopathology) by using data from real experimental studies. Our findings showed that the statistical power of the EAMA could be considered in line with the one of OECD standardized tests such as the AMA and the LAGDA which represent a step forward towards further regulatory acceptance of this test design. Our results also showed that the LAGDA is statistically more powerful. However, the magnitude of the observed difference does not justify the submission of a LAGDA to address concerns identified in an AMA, strengthening the conclusions reached by other authors that the findings from the AMA may be sufficient to conclude on the identification of endocrine disruptors through the thyroidal modality. This work is presented under the sole responsibility of the authors who are currently employed with the European Food Safety Authority (EFSA), and may not be considered as an EFSA scientific output. The positions and opinions (if any) presented in this article are those of the authors, and do not necessarily represent the views of EFSA.

1.12.P-Tu132 How to Break the Silos and Strengthen the Weight of Evidence: An Example

Maria Arena¹, Simone Rizzuto², Aude Kienzler² and Laura Villamar-Bouza¹, (1)European Food Safety Authority, Italy, (2)European Food Safety Authority, Parma, Italy

In the context of the assessments for endocrine disruption for the environment, the weight of evidence is fundamental when trying to consider all the available data together and reach a robust conclusion on whether the criteria as laid down in the Regulation 605/2018 are met or not. The strength of the weight of evidence often depends on the data richness on one side, and on the knowledge and gaps therein that may

exist for specific cases on the other side.

The ED assessment of pesticide active substances is performed both for mammalian and non-mammalian species and two separate conclusions are normally drawn since one considers specific data on mammals and the other consider data on fish for Estrogen, Androgen and Steroidogenesis (EAS) modalities and amphibians for the Thyroid modality. Although the ECHA/EFSA Guidance on the hazard identification of endocrine disruptors suggest a holistic approach, this approach is rarely followed, especially for the identification of EDs in humans. In addition, although the endocrine system is known to be conserved across vertebrates, mammals and non-mammalian species may show different sensitivity and therefore a different outcome of the assessment may be possible. The experience and knowledge gained from EFSA so far have shown, however, that in the case of substances suspected to be anti-androgens a conclusion for non-mammalian species may be challenging since the available study designs with fish do not allow to clearly detect anti-androgens. The use of mammalian and non-mammalian data in a holistic manner has resulted in strengthening the weight of evidence and has allowed to reach a conclusion for the environment which would have not been possible if data on non-mammalian species had not been considered.

The poster will show one case of a holistic approach where mammalian and non-mammalian data have been used together. The example will show how data for endocrine activity and adversity for mammalian and non-mammalian species have been assessed and consider together in the Mode of action analysis to strengthen the weight of evidence and conclude the substance under assessment was an endocrine disruptor.

1.12.P-Tu133 Avoiding Unnecessary Animal Testing: Applying a Weight of Evidence Approach to Challenge Regulatory Decisions on Endocrine Disrupting Potential

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A substance evaluation under Regulation (EC) No. 1907/2006 was initiated by the European Chemicals Agency (ECHA) on a plasticizer used as phthalate substitute, under special focus due to its widespread use in plastics, including PVC applications. The substance was assessed as part of ECHA's broader investigation into PVC additives and an "Assessment of Regulatory Needs" (ARN). At the time of the evaluation, the dossier included the outcomes from a Fish Sexual Development Test (FSDT, OECD TG 234) in which no significant effects on sex ratio or vitellogenin concentration had been observed. Despite the negative outcome for endocrine disrupting (ED) properties in the FSDT, ECHA initially requested the conduct of an extended one-generation reproduction study (EOGRT) in Medaka or zebrafish (OECD TG 240), citing concerns that the substance may possess endocrine-disrupting (ED) properties. In response to the draft decision, a weight of evidence (WoE) analysis was conducted, incorporating *in silico*, *in vitro*, and *in vivo* data from mammalian and aquatic studies. While some *in vitro* assays showed weak estrogen receptor activity, at concentrations far in excess of aqueous solubility, the WoE analysis indicated that the substance does not fulfil the criteria required for a substance to be classified as an endocrine disruptor. Specifically, there was no evidence for an adverse effect in an intact animal via an endocrine mode of action. In this case, the WoE assessment, coupled with expert opinions and ethical concerns based on the 3R principles (replacement, reduction, and refinement of animal testing), led to a successful appeal with the competent authority agreeing that the fish extended one-generation reproduction toxicity study was not required.

This underscores the importance of evidence-based decision making and highlights the need for improved guidance in interpreting and using WoE assessments in regulatory decision-making for endocrine disruption.

1.12.P-Tu134 Navigating New Challenges and Divergences in Endocrine Disruptor Assessments Under European Regulations

Chloe Eastabrook, Gareth Le Page and Charles Hazlerigg, Enviresearch, United Kingdom

The recent release of ECHA's updated Classification, Labelling and Packaging (CLP) guidance introduces endocrine disruptor (ED) hazard classification. Although this updated CLP guidance works in conjunction with ED guidance already available for pesticides and biocides from ECHA EFSA (2018), the addition of ED hazard classification into CLP creates new challenges at every stage of the regulatory process. As CLP regulation does not require any new testing to be conducted to conclude a hazard classification for a substance, this updated guidance highlights the crucial role of Weight-of-Evidence (WoE) assessments in determining a chemical's ED properties. This is because of the CLP's dependence on available data, which when sourced from the open literature can vary significantly in reliability and quality. Consequently, there's a pressing need for robust WoE methodologies and tools, especially for data-poor chemicals that

lack regulatory studies or naturally interact with the endocrine system. Challenges with this updated CLP guidance also arise where it diverges from the ECHA EFSA (2018) guidance for pesticides and biocides. While the updated CLP guidance relies heavily on the pesticide and biocide guidance in some places, there are some major divergences between their approaches and classification of ED. For example, CLP introduces a new classification option of suspected ED, in addition to known or presumed ED in the pesticide and biocide guidance, but there is no clear guidance or worked examples provided on how to differentiate these options. The strength of evidence needed to distinguish between categories 1 and 2 will likely vary chemical to chemical, as data sufficiency will impact the WoE assessment. Additionally, the updated CLP guidance supports different tools and encourages classification based on New Approach Methodologies (NAMs) and other predictive data to be included in the WoE, as additional testing is not required. In this poster presentation these approaches to assessing ED under CLP, compared to the pesticide and biocide regulations, will be explored, with a focus on the use and role of WoE within each.

1.12.P-Tu135 A Word of Caution on the Use of Poorly Documented Adverse Outcome Pathways for Endocrine Disruption Assessment

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The adverse outcome pathway (AOP) concept was developed to organize available information describing how the interaction of a chemical with a molecular target (the Molecular Initiating Event, MIE) can result in an adverse outcome (AO) in individuals and, by extension, in a population. In an AOP, the MIE is causally related to the AO through a series of key events (KEs) linked by key-event relationships (KERs). As they are based on a biologically plausible suite of KEs, AOPs can be used in weight-of-evidence approaches to identify endocrine disruptors. An AOP that is sufficiently populated with substance-specific data allows establishing plausible links between KEs leading to an individual-level AO. AOPs should, however, be used with caution for insufficiently documented substances for which only limited in vitro data or in vivo biomarker responses are available. In such cases, AOPs may be misinterpreted as there is an expectation that a MIE or any (early) KE will automatically induce the entire cascade of events always leading to the AO. In reality, many factors can interfere with KERs, so that an AO may not occur even if a MIE has been identified and effects on KEs are empirically observed. For example, a certain level of exposure at the target site is required for a MIE to generate a KE, and for a KE to be sufficiently impacted to activate the next KE. There can also be homeostatic mechanisms, feedback loops, or non-responding KE(s) that may interrupt the cascade of KEs, so that the AO does not manifest. In fish AOPs (e.g. AOPs 23, 25 and 30), the population-level AO invariably refers to a declining population trajectory. There are, however, multiple modeling and comparative studies showing that traits that are sensitive to toxicants, e.g. reproduction, can be of low impact on population growth. Also, field studies have shown no correlation between the exposure to estrogenic compounds and the density and self-sustainability of feminized fish populations. Overall, as there are multiple reasons for an AO not to occur as the result of the interaction of a chemical with a molecular target or a KE, AOPs should not be used as tools to predict individual-level AO from (sub)individual responses. To do this, detailed quantitative AOPs with established potency thresholds for each KE and AO predictions are needed. Higher-tier approaches such as population modelling, or targeted field studies would be required to extrapolate the AO from individual to population.

1.12.P-Tu136 The SETAC MAPPED Workshop: Population Modelling to Assess the Effects of Endocrine Disruption on Freshwater Fish

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The SETAC workshop MAPPED aims to improve the applicability of Models to Assess the PoPulation relevance of effects of Endocrine Disruptors (ED), for fish and amphibians. The poster will present the current status of the fish populations subgroup's homework after the first workshop in September 2024 with a second workshop scheduled to take place in June 2025. The working group has set out to answer two main questions: First, how can we best define focal fish species most appropriate for modelling the effects of ED substances at the population level and second, in what ways can /should these models be used? In relation to the first question, a life history and behavioural trait database will be established using

information from the AddmyPet and FishBase databases and this dataset will be used to group freshwater fish according to their trait combinations, with the aim of selecting a set of optimally 5-10 focal species representing the diversity of fish at risk to ED. The selection of focal species will also consider additional characteristics such as geographical distribution, availability of developed population models or data sets for model development and testing. The modelling topic will address the questions of what data to include in the population models, the definition of environmental scenarios, the use of the normal operating range, model validation and how to assess relevance of population level effects. The findings from this work will be published in a SETAC journal.

1.12.P-Tu137 A 2024 Inventory of Test Methods Relevant to Thyroid Hormone System Disruption for Human Health and Environmental Regulatory Hazard Assessment

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Thyroid hormone system disruption (THSD) is a growing concern in chemical hazard assessment due to its impact on human and environmental health and the limited validated methods available for assessing the THSD potential of chemicals. In particular, the general lack of validated in silico and in vitro methods for assessing THS activity is of high concern. In this work, we provide an inventory of test methods relevant to THSD. Building on the Organisation for Economic Co-operation and Development (OECD) Guidance Document 150 and recent international developments, we highlight progress in in silico and in vitro methods, as well as in vivo assays. The provided inventory categorizes available methods according to the levels of the OECD Conceptual Framework, with an assessment of the validation status of each method. At Level 1, 12 in silico models that have been statistically validated and are directly related to THSD have been identified. At Level 2, 67 in vitro methods have been listed including those assessed in key initiatives such as the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL) validation study to identify potential thyroid disruptors. At Levels 3-5, THSD-sensitive endpoints are being included in existing fish-based OECD Test Guidelines to complement amphibian assays. In total, the inventory counts 108 entries comprising established methods (e.g., OECD Test Guidelines) as well as citable methods that are under further development and in some cases are ready for validation or in the initial stages of validation. This work is part of the Partnership for the Assessment of Risks from Chemicals (PARC), an EU-wide research and innovation programme funded by Horizon Europe and focused on bringing the science to the regulators, among others, in the area of endocrine disruption. To this aim it aligns work on adverse outcome pathway development for endocrine disruption with work on the development of AOP-based approaches for endocrine disruption. Several projects in PARC contributed to this work aiming to support the ongoing development of strategies for regulatory hazard assessment, such as integrated approaches to testing and assessment (IATAs), for endocrine disruptors, addressing critical gaps in the current testing landscape for THSD in both human and environmental health contexts. This project has received funding from the European Union's Horizon Europe Research and Innovation Programme under Grant Agreement No 101057014 (Partnership for the Assessment of Risks from Chemicals [PARC]) and from the University of Antwerp Research Fund (project 44602) and the Belgian Federal Public Service Public Health, Food Chain Safety and Environment, DG Environment, in the framework of the National Action Plan on Endocrine Disruptors (NAPED, DGEM/DPPC/MJE/23079). Views and opinions expressed are those of the author(s) only and do not necessarily reflect those of the European Union or the Health and Digital Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

1.12.P-Tu138 Mapping, Identification, and Assessment of Endocrine Disrupting Chemicals

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Environmental authorities face significant challenges in managing the risks posed by numerous endocrine-disrupting chemicals (EDCs). Advanced computational ecotoxicology tools, such as quantitative structure-activity relationships (QSARs), are essential in evaluating environmental hazards. To make toxicity data predictions practical for regulatory frameworks, it is crucial to transform this data into coherent, synthesized information through classification and grouping of substances. Under the PARC project's umbrella, we have mapped over 7,000 endocrine-disrupting (ED) substances. The database includes predicted data on substances' intrinsic physico-chemical properties, monitoring data, NORMAN prioritization indicators, ECHA's article service life, consumer uses and widespread uses, process categories, environmental release categories, and over 20 endocrine disruption-related model predictions. We conducted a comprehensive mapping to assess several key aspects. These included evaluating the coherence among different model predictions as well as examining the relationships between various modes of action and the substances. We explored endocrine-disrupting (ED) activities and quantified the number of substances associated with each ED route. This analysis was performed to emphasize the importance of in vitro data within regulatory frameworks, given that regulations predominantly consider pNEC values for daphnia, fish, and algae. Mapping results will serve as a baseline reference for understanding ED activity across various chemicals. This dataset not only enhances our ability to identify and categorize substances based on their potential risks but also provides a valuable baseline for future research and regulatory efforts.

1.12.P-Tu139 Weight of Evidence Endocrine Disruptor Assessments for Hydrocarbon UVCBs

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New hazard classes are being introduced under the Classification, Labelling, and Packaging (CLP) regulations to address endocrine disruption (ED). ED assessments are complex and data-intensive for mono-constituent substances with standardised datasets but the current CLP guidance does not fully consider the challenges for assessing more complex UVCBs (substances of unknown or variable composition, complex reaction products, or biological materials). Currently, classification assessments for ED will largely focus on individual constituents, which implies that there is sufficient data on all components to adequately characterise the ED hazard. Hydrocarbon UVCBs can encompass thousands of different constituents where some of them can be unknown and uncharacterised. A pragmatic approach that considers both the available data for the UVCB whole substance and for the constituents is therefore needed to assess the ED potential of UVCBs. Here, we applied a tailored approach for conducting an ED assessment for hydrocarbon UVCBs. To limit the number of constituents in this first assessment, hydrocarbon constituents at potential concentrations of ?1% in at least one petroleum substance were selected from a list of hydrocarbons with potential endocrine activity and/or reproductive toxicity. Constituent data were then screened for reliability (e.g. in vitro data assessed as unreliable if constituent properties [e.g. volatility, solubility] are not considered). Following this exercise, a stepwise evaluation was followed: i. Data gathering: Collate information from regulatory sources, open databases, and abstract screening of peer-reviewed articles; ii. Initial data summary: Summarise endocrine activity data and in vivo data from studies (with mammals and non-target vertebrates) identified in regulatory sources or ED databases; iii. Vulnerability assessment: Rank constituents based on ED concern, proposing an initial minimum threshold for ED classification; iv. Weight-of-evidence assessment: Begin with the highest-ranked constituents, phasing the analysis so that when a definitive conclusion can be drawn from a limited dataset (e.g. EATS [estrogen, androgen, thyroid and steroidogenesis]-mediated effects), efforts can be focused to other constituents. Our approach aims to enable initial ED conclusions for hydrocarbon UVCBs and potentially support the development of evidence-based read-across strategies for hydrocarbon constituents.

1.12.P-Tu140 Endocrine Disruption Assessment of UVCBs (Substances of Unknown or Variable Composition, Complex Reaction Products or Biological Materials): A Case Study Using Grease Thickeners

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New hazard classes for endocrine disruption (ED) have been introduced under Classification and Labelling (CLP) regulations. Mixture rules are expected to be applied to UVCBs (substances of unknown or variable composition, complex reaction products or biological materials), so that ED classification is based on the most conservative outcome for the whole substance and relevant constituents present above a concentration limit (e.g. 0.1%). An approach for ED assessment of UVCBs has been developed for petroleum substances: gather available data (on whole substance, substance composition and individual constituents), consider whether classification is possible based on existing classifications for individual constituents or whole substance data, and finally evaluate data for individual constituents. This general ED assessment approach for UVCBs has been adapted for use in the initial prioritisation of grease thickeners. Most grease thickeners are either registered at lower tonnage levels, or using read across from similar substances, so ED assessment based on whole substance data is not possible as no or limited relevant whole substance data are available for human health or the environment. Therefore, the prioritisation for ED assessments has focussed on relevant constituents. Compounds relevant for the ED assessment were identified, including the substances themselves and the constituents and impurities therein, immediate transformation products and read across source substances. This expanded the initial list of 50 substances in the grease thickener portfolio to 191 compounds requiring ED evaluation. Substance characterisation is ongoing for some complex substances, which could expand the list further. No constituents have yet been concluded for ED and most have limited relevant data. Grease thickener substances consist primarily of metal salts of carboxylic acids, with differences in chain lengths, functional groups and metal cations, as well as substances with more complex structures. Therefore, the reliability assessment and interpretation of data is likely to be complex, especially as some substances are poorly soluble and metal salts are outside the applicability domain of many *in silico* models. This case study demonstrates some of the challenges associated with ED assessments for these substances and offers perspectives and strategies for dealing with ED assessments of complex substances. The authors would like to thank the European REACH Grease Thickeners Consortium (ERGTC) for sharing this work.

1.12.P-Tu141 Validation of the tFET: Enhancing the OECD 236 Fish Embryo Toxicity Test with Novel Endpoints to Assess Thyroidal Hormone System Disruption by Chemicals

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Endocrine-disrupting chemicals pose significant risks to human and environmental health, as even low concentrations can severely imbalance physiological homeostasis in organisms, affecting both individuals and populations. Current ecotoxicological assessments, such as the OECD 231 Amphibian Metamorphosis Assay or OECD 248 *Xenopus* Eleutheroembryonic Thyroid Assay (XETA), evaluate thyroid-disruptive properties but require testing on protected animal life stages, raising ethical concerns. An implementation of further endpoints surrogate for thyroidal disruption into existing TGs such as the OECD 236 Fish Embryo Acute Toxicity Test (FET), designed as an acute toxicity test in fish but limited to non-protected life stages bares the potential to reduce the need for multiple tests and hence to spare resources and animals in line with the 3Rs principle and sustainability. In a collaborative project of academic and industry partners (ERGO Project), an expanded thyroid FET (tFET), with novel endpoints that assess disruptions of the hypothalamic-pituitary-thyroid axis has been designed and shall be validated for regulatory use in ecotoxicological hazard assessment. Participating in the validation process of this modified OECD236 guideline, we will validate the test design and selected surrogate endpoints, e.g. the posterior swim-bladder inflation, changes in the eye histopathology and measuring of thyroidal-related hormone levels in exposed organisms. To minimize systemic toxicity interference, chemical concentrations will be capped at the maximum tolerated dose, defined by the 96-hour LC10 following OECD 236 guidelines. Initial progress includes the identification of potentially suitable substances, pretesting, and determination of appropriate concentration ranges based on 96-hour LC10 values.

1.12.P-Tu142 The Zebrafish Extended One Generation Reproduction Test (ZEOGRT) – Experiences and Current Results from the Validation Exercise

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For the identification of endocrine disrupting properties of chemicals, the WHO/IPCS definition requires a causal link between an endocrine mechanism of action and an adverse effect with population relevance. Within the OECD Conceptual Framework for endocrine testing and assessment, multi-generational tests are assigned to provide data over more extensive parts of the life-cycle of the organisms. In the aquatic environment, fish populations are considered as main targets of endocrine acting compounds. The adopted OECD guideline 240, i.e. the Medaka Extended One Generation Reproduction Test (MEOGRT), was designed and validated for a single test species only. On the initiative from Germany, a similar test approach with zebrafish (*Danio rerio*) as a further species was developed. Both protocols include the exposure of a parental generation (adult fish), a full Filial 1 (F1) generation (from egg to adult) and a Filial 2 (F2) generation (until hatch of embryos). The development of the Zebrafish EOGRT protocol was placed on the OECD workplan in 2016. The initial phase of the validation process, started in 2018, included four ZEOGRT studies to confirm the applicability of the study protocol. The test substances applied were tamoxifen-citrate (TMX), prochloraz (PRO), dienogest and dexamethasone. The effect results obtained for TMX and PRO were in good compliance with available literature and it was possible to build a bridge to the validation studies conducted for the MEOGRT. The results and evaluations were summarised in a validation report for Phase I and presented to OECD. In the second part of the validation, started in 2022, international cooperation partners were identified to apply the protocol to their labs and to prove the reproducibility of the test design. In total, one additional study with TMX and two more studies with PRO were successfully conducted. These data will be used to allow an evaluation of inter-lab variability to be presented in a second validation report. To extend the data set, available control data from industry study will be used. The presentation gives an overview on the final results obtained and shows a comparison of effect concentrations derived from the different lab studies. The available results confirm the applicability of the ZEOGRT test protocol. The data obtained are valuable to identify sensitive endpoints and gain mechanistic information used for an endocrine assessment.

1.12.P-Tu143 An Extended Amphibian Metamorphosis Assay (EAMA) with the Reference Compound Propylthiouracil (PTU)

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The Amphibian Metamorphosis Assay (AMA) is a key in vivo screening assay intended to identify substances which may interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis. The Extended Amphibian Metamorphosis Assay (EAMA) is a recently proposed, modified design that was created to better evaluate thyroid-related endpoints using elements from the Larval Amphibian Growth and Development Assay (LAGDA); specifically, the development endpoint as a time-to-stage evaluation instead of stage determination at study day 21. There are several advantages to this change in design vs. the AMA. For instance, the robustness of the statistical and histological analyses is improved and extending to NF stage 62 encompasses exposure during metamorphic climax, when thyroid hormone plasma concentrations dramatically increase. While regulatory studies have been performed with this extended design, no studies with reference chemicals have been conducted. Therefore the goal of this study was to evaluate EAMA endpoints after exposure to a reference toxicant and compare responses of thyroid-mediated endpoints vs. the fixed-time design. To this end, two extended AMA studies were conducted with propylthiouracil (PTU). Study #1 utilized the concentration range from the OECD test guideline 231 validation exercise: 2.5, 5.0, 10, and 20 mg/L and a dilution water control. A follow-up study employed a 40 mg/L treatment group and a dilution water control. Statistically significant delays in development were observed between the control and the 20 mg/L treatment group at test termination. Metamorphic developmental arrest was observed at the 40 mg/L treatment group, as tadpoles did not develop past NF stage 56. In addition, statistically significant increases in wet body weight, snout-vent length (SVL), and hind limb length normalized by SVL were observed in 40 mg/L-exposed tadpoles compared to the control. Findings in the thyroid glands at 10, 20, and 40 mg/L included concentration-dependent increases in the prevalence and severity of follicular cell hypertrophy and follicular cell hyperplasia as well as decreased colloid. Thyroid hypertrophy was observed at 20 and 40 mg/L. The results were in line with the expected outcome based on the mode of action of PTU as a thyroid hormone synthesis inhibitor and provide data for validation of the EAMA as a reduction and refinement alternative method to the AMA or the LAGDA.

1.12.P-Tu144 Advancing In Vitro Assessment of Iodide Uptake Inhibition: Integrating a Novel Biotransformation Pretreatment Step

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Thyroid Hormones (TH) are essential for vertebrate development, growth, and metabolism. The limited knowledge on the TH-disrupting potential of anthropogenic chemicals emphasizes the need for improved methods to assess their impacts on TH homeostasis. Inhibition of the Sodium Iodide Symporter (NIS), a key step in TH production, has been identified as a crucial molecular initiating event affecting the TH system and related adverse effects in various species. The role of NIS has also been implicated in thyroid and other cancers. NIS transported I⁻ radioisotopes are used to diagnose, treat, and monitor thyroid pathologies and NIS can also be used as a diagnostic transgene. Thus, compounds interfering with NIS could affect not only cancer development but also the sensitivity of its diagnosis and its treatment efficiency. We have developed in vitro bioassays to evaluate the effects of chemicals on iodide uptake mediated by NIS, using two stably transfected human cell lines overexpressing human NIS. Their performance was compared against FRTL-5, a rat thyroid cell model with endogenous NIS expression. We have characterized the dose-response relationship for 23 model compounds using a non-radioactive colorimetric Sandell-Kolthoff reaction to detect the uptaken iodide levels along with detailed cytotoxicity assessment. The observed effects were also compared to those detected by other in vitro models in the literature, mostly using radioactivity-based assay. To enhance the physiological relevance, we incorporated an external biotransformation system (BTS), optimized for live-cell use without inducing cytotoxicity or assay interference. The findings show that in vitro metabolic capacities could mitigate the inhibitory effects of certain compounds on iodide uptake and thus metabolic modification of these compounds. Moreover, our experimental results were corroborated by in silico prediction and published in vivo data, at least for the compounds that showed no bioavailability issues. However, there were some limitations for hydrophobic chemicals. To reflect this, we currently suggest implementing the biotransformation-augmented NIS assay only for compounds with a log K_{ow} up to five. Noteworthy, we recommend that potential future users of the BTS-augmented NIS assay always include a set of relevant controls, as these are essential for accurate data interpretation, detecting bioavailability-related effects, and understanding the assay's potential applicability range.

1.12.P-Tu145 Assessment of the In Vitro Test Guideline OECD 455 for Detecting Estrogenic Activity using Animal-Free Conditions and Animal-Free Metabolization Systems

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Regarding cell-based in vitro assays, controversial animal-derived components like the fetal bovine serum (FBS) collected from non-anesthetized calf fetuses and the phenobarbital/β naphthoflavone induced liver homogenate fraction (S9) collected from rats are frequently used. These components, used for promoting cell growth and for simulating biotransformation processes respectively, are known for interacting with test substances, being potentially contaminated by microorganisms and causing increased variability within the assays due to their undefined composition. To decrease animal cruelty and provide stable comparable assay results efficient alternatives such as chemically defined matrices should be considered. Contributing to this, the aim of this study is to develop the in vitro assay ER⁺ CALUX® for detecting estrogenic activity towards animal-free conditions and the incorporation of animal-free metabolization systems. This will be established by evaluating the lowest amount of FBS needed for a valid ER⁺ CALUX® test result applying the manufacturers validity criteria established for the standard, FBS containing procedure. After evaluating the outcome of this approach, the sensitivity of the optimized assay will be tested by using model substances and groundwater samples from the project gwTriade, a project aiming to establish an integrated assessment of groundwater systems. Therefore, different scenarios will be compared regarding their performance and comparability based on the in vitro test guideline OECD 455: (1) The currently standardized FBS-containing medium (2) The chemically defined medium without FBS and (3) The chemically defined medium with the optimized amount of FBS from the improvement approach. Additionally, those approaches will be tested with the supplementation of animal-derived S9 and biotechnological, animal-free S9 (ewoS9). At present, the addition of 0.1 % FBS to chemically defined medium for conducting a valid in vitro ER⁺ CALUX® seems promising. If evaluated as reproducible in further experiments, this would result in the use of 98 % less FBS per assay plate contributing to a successful reduction in animal components following the 3R's principle of replacing, reducing and refining the use of animals in scientific research. Consequently, this could also contribute in achieving less variability within the assays, providing better reproducible and comparable results on estrogenic activity in environmental samples.

1.12.P-Tu146 Zebrafish Hepatic 3D Spheroid Models for High-Throughput Screening of Endocrine Disruptors Promoting Steatotic Liver Disease

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is one of the most common liver disorder, affecting approximately a quarter of the world's population. Growing evidence indicates that exposure to endocrine-disrupting chemicals (EDCs) may contribute to the initiation and/or progression of MASLD, either directly or by amplifying the effects of high-fat diets, genetic predispositions, and lifestyle factors. For rapid screening and identification of relevant EDCs and mixtures promoting MASLD, a three-dimensional (3D) in vitro spheroid system derived from the zebrafish (*Danio rerio*) liver cell line (ZF-L) was optimized for high-throughput (HT) screening (384-well plate format) and chronic testing (up to 14 days of exposure). The spheroids showed good sphericity and viability in a 384-well plate after 16 days, suggesting the suitability of this model for HT chronic toxicity testing. A MASLD-relevant test battery is currently under development, including assays for measuring cytotoxicity (LDH and ATP), lipid storage and metabolism (Nile red and TAG), oxidative stress (ROS) and cell morphology. Three model compounds known to affect lipid metabolism, dichlorodiphenyldichloroethane (ppDDE), perfluorooctanoic acid (PFOA) and triphenyl phosphate (TPP), will be used to validate the test system. High-throughput screening will then be conducted to identify and prioritize relevant EDCs from a large list of the MASLD cohort studies. This novel in vitro high-throughput test system as a new approach methodology (NAM) is foreseen to significantly reduce testing needs in future EDC-MASLD research.

1.12.P-Tu148 Morphological Changes in Brain Development of Zebrafish Embryos After Exposure to Thyroid Hormone System Disrupting Chemicals

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Thyroid Hormone System Disrupting Chemicals (THSDCs) are known to affect the development of the nervous system in vertebrates by interfering with thyroid hormone system (THS) during early-life development. Indeed, THs play a crucial role in brain development, including the differentiation of various brain cell types, such as oligodendrocytes. As a result, assessing THS disruption effects on brain development is a significant concern for environmental and human health safety. We are currently investigating the effects of THS disruption using two different approaches, both involving zebrafish embryos. Fish embryo models are widely used in (eco)toxicological research to investigate the impact of chemicals on whole organisms, as they represent a good alternative to mammal and adult fish testing according to 3R. Our first approach involves a triple-transgenic zebrafish line, which allows for the quantification of three different brain cell types and thereby the detection of developmental neurotoxicity (DNT). In this line, neurons, astrocytes and oligodendrocytes express three distinct fluorescent proteins, driven by the regulatory sequences of enolase 2, glial fibrillary acidic protein, and myelin basic protein, respectively. Previous studies have shown alterations in oligodendrocytes differentiation following exposure to T4 and TH synthesis inhibitors in zebrafish embryos. Consequently, we are investigating exposure to different THS disruption mechanisms of action, that likely result in structural alterations of oligodendrocytes, and potentially affect neurons and astrocytes as well. Secondly, histopathology analysis of specific brain structures is conducted in embryos exposed to THSDCs. Preliminary data from the ERGO project show that THSDCs impair brain development of zebrafish exposed until the juvenile stage. We expect effects to be visible already during embryogenesis and are therefore performing histopathological analyses of embryos exposed to the same set of THSDCs. Distinct histopathology effects may facilitate the identification and interpretation of DNT induced by THSDCs. These new investigative approaches are combined with specific TH-related endpoints as TH levels and thyroid follicle morphology to assess sensitivity and specificity of those novel endpoints. In summary, we believe that new endpoints focusing on DNT could be used for THS disruption assessment.

1.12.P-Tu149 Comparison of Thyroid Disruption Potentials of Urinary Plasticizer Exposure Profiles among Children of Four Asian Countries using Larval Zebrafish

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Due to different regulatory environments, exposure profiles of major plasticizers among children differ by country. However, the toxicological implications of such variation are elusive because of the complexity of toxicity interactions among the plasticizers. In this study, based on the urinary concentrations of major plasticizer metabolites in the children of South Korea (n=87), Thailand (n=110), Indonesia (n=92), and Bangladesh (n=100), a 7-day embryo-larval zebrafish exposure experiment was designed for thyroid hormone disruption-related endpoints. For this purpose, daily intakes (DI, $\mu\text{g/kg bw/day}$) of parent compounds (DMP, DEP, DiBP, DnBP, DEHP, DiNP, DEHTP) were estimated with creatinine adjustment. Then, this information was used to estimate their corresponding concentration in the fish plasma, and their concentration in the fish exposure media. The calculating method is as follows. C_p is the blood plasma concentration resulting from a known exposure dose (E). $C_p (\text{mol/L}) = E[\text{mol/kg bw}] \times (t_{1/2}) / 0.693 \times 1 / (V_d[\text{L/kg bw}])$ Compared to the SC group (DMSO 0.01%), zebrafish exposed to chemical mixtures representing Bangladesh exposure showed significant reductions in thyroid hormone regulatory genes, synthesis-related genes, and metabolism-related genes. Similarly, the fish exposed to the Indonesian mixture demonstrated a significant decrease in thyroid hormone regulatory gene expression. The mixtures from Korea and Thailand did not exhibit significant alterations. The current observations suggest that the higher quantities of detected plasticizers and composition of traditional plasticizers in Bangladesh and Indonesian mixtures contribute to significant thyroid disruption compared to those of South Korea and Thailand. Further research is warranted to identify the chemicals that explain observed outcomes, providing insights into risk mitigation and regulatory strategies for vulnerable populations. This work was supported by the Research Grant from Seoul National University(900-20240102) This work was supported by the Ministry of Education of the Republic of Korea and the National Research Foundation of Korea (BK21 FOUR 5199990214126)

1.12.P-Tu150 New Data To Determine The Thyroid Mode Of Action Covered By The Xenopus Eleutheroembryonic Thyroid Assay

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In June 2019, the OECD validated the Xenopus Eleutheroembryonic Thyroid Assay (XETA) in the test guideline program in order to support the identification of thyroid active chemicals. Since its validation, the XETA is used for the evaluation of the thyroid modality for biocides and pesticides. To date, we could estimate that more than 30 chemicals were assessed for the thyroid modality using a weight of evidence methodologies including data from a XETA. Following the validation exercise, several studies have been conducted to expand the dataset of reference chemicals. To enhance the dataset of chemicals tested in both amphibian assays, 17 additional chemicals with available Amphibian Metamorphosis Assays were evaluated. A separate study investigated 3 plasmatic transport inhibitors to generate data on this mode of action, which had previously been insufficiently explored. As part of the EU Horizon 2020 program under the ATHENA project, 8 thyroid-active chemicals identified through in vitro screenings were tested. Furthermore, a new study was conducted with 12 chemicals selected based on their specific MoA, further enriching the database for these particular mechanisms. When added to the number of chemicals included in the validation, 56 reference chemicals have now been tested in the XETA. Overall, the various studies demonstrate that the XETA effectively detects plasmatic transport inhibitors as well as thyroid receptor agonists and antagonists. Deiodinase inhibitors and transmembrane transport inhibitors were also identified using the XETA; however, these MoAs were represented by only a limited number of substances, necessitating further confirmation with additional test chemicals. While TPO inhibitors showed positive results in the XETA, for some of them their activity did not align with the expected MoA, further investigations are in progress to explore the physiological mechanisms underlying these results. Additionally, all tested NIS inhibitors were inactive in the XETA, indicating that this particular MoA is not captured. The XETA is an ethical and novel short-term screening assay that could efficiently be use in a weight of evidence approach for the thyroid modality. Knowing that substances that inhibit NIS are not detected and that TPO inhibitors are not adequately identified, the result of the XETA could be combined with in vitro TPO and NIS assays (in the process of OECD validation) for comprehensive coverage of all thyroid mechanisms of action. The authors thank Croplife Europe and the EU Horizon 2020 program, ATHENA project, grant number 825161 for financial support.

1.12.P-Tu151 TG252 the Rapid Estrogen ACTivity In Vivo (REACTIV) Assay – Results from the OECD Validation and Next Steps

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Huge efforts are currently underway in Europe, Asia and North America to identify chemicals that

interfere with estrogenic signalling. With increasing pressure in Europe to reduce animal testing and test facilities which are saturated with requests for traditional fish tests, both chemical manufacturers and evaluating authorities are beginning to look towards new approach methodologies (NAMs). Eleutheroembryos, aquatic vertebrates, not compliant with the European definition of a laboratory animal, provide physiologically relevant whole animal data while allowing for rapid testing of large numbers of chemicals at a greatly reduced cost. The Rapid Estrogen ACTivity In Vivo (REACTIV) assay is a 24 h assay using transgenic medaka fish eleutheroembryos harbouring the choriogenin h promoter driving expression of gfp. Choriogenin plays an essential structural role in egg formation and responds directly to estrogen axis signalling, like the highly related vitellogenin biomarker. The OECD interlaboratory validation exercise showed highly reproducible results. The transferability of the assay was well demonstrated. All of the inert chemicals which were tested were correctly identified as inert, indicating that the REACTIV assay is highly selective. Data generated for 12 chemicals which act on the estrogen axis via differing modes of action clearly indicated the ability of the REACTIV assay to identify chemicals acting on estrogen axis signalling, either at the receptor level or on downstream steroidogenesis. Only the 5 α -reductase inhibitor, dutasteride, gave equivocal results. Following the successful interlaboratory validation exercise the REACTIV assay was ratified by the OECD as a test guideline (TG 252). The REACTIV assay is a rapid 24 h assay that allows the quantification of estrogen axis activity. The eleutheroembryonic life stages on which the assay is based are in line with the three R s principle of animal replacement. Although the results for 5 α -reductase inhibitor, dutasteride, gave equivocal results, this mode of action is well covered by the androgen/ anti-androgen sensitive RADAR assay (TG 251). Therefore, combining the REACTIV and RADAR assays with existing data from mammalian tests as well as in vitro and/or in silico could allow the evaluation of estrogen, androgen and steroidogenesis activity of chemicals without the use of laboratory animals, we suggest how they could be integrated into regulatory decision trees and IATAs to achieve this aim.

1.12.P-Tu152 Identification of Chemical Substances with Ecdysteroid Activity in *Daphnia magna* using in Silico Analysis, in Vitro and in Vivo Assays

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Ecdysone is an essential hormone regulating development and molting in arthropods including *Daphnia magna*. While several ecdysone receptor (EcR) agonists used as insecticides (e.g. tebufenozide) are already known, it is possible that other chemical substances also have ecdysteroid activity. The Extended Tasks on Endocrine Disruption 2022 (EXTEND2022) implemented by the Ministry of Environment, Japan, have developed two-tiered framework for testing and assessment where candidate test chemicals are selected based on literature review and subjected to Tier 1 in vitro reporter gene assay and short-term in vivo screening assay to evaluate endocrine disrupting effects. To efficiently detect ecdysoteroid activity of chemicals, we selected candidate chemicals based on the result of in silico analysis from the literature (Mellor et al., 2011) and conducted in vitro reporter gene assay and short-term in vivo assay using *Daphnia magna*. Antibiotic chloramphenicol and anti-inflammatory drug aminopyrine were selected from the in silico results because they have diacylhydrazine and methylene- β -lactam motif, respectively, which are essential structure for receptor binding site, however, no EcR transactivation was shown in the reporter gene assay. The short-term in vivo assay demonstrated the significant decrease in the number of molts from offspring to mature adult (8 days) exposed to these chemicals as well as EcR agonist. To distinguish the effect from general toxicity and disruption in ecdysteroid signaling pathway, chitin degrading enzyme chitinase released into test solution was measured as biomarker specific to molting inhibition. Although EcR agonist such as 20-hydroxyecdysone, tebufenozide, and ponasterone A demonstrated a significant decrease in chitinase activity compared to control and a positive correlation between chitinase activity and the number of molts, the candidate chemicals showed no correlation. Therefore, another in silico analysis approach such as molecular docking simulation is needed for prioritizing candidate chemicals. Moreover, by integrated assessment of the data from in silico analysis, in vitro and in vivo assays with biomarkers could accurately identify chemicals with ecdysone activity.

1.12.P-Tu153 Investigation into the Mechanism(s) of Chronic Copper Effects on Zebrafish Reproduction

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Chronic exposure to copper (Cu) has been demonstrated to reduce fecundity in several species of fish, but

the mechanism(s) causing this reduction are not well understood. We hypothesized that reduced fecundity upon Cu exposure could be caused by either an endocrine mediated process or direct oxidative damage to either the gonads and/or liver. To test this hypothesis, we exposed reproductively active zebrafish (*Danio rerio*) to Cu concentrations of 0, 6.7, 20, and 60 $\mu\text{g L}^{-1}$ for 21 days. We assessed both endocrine and non-endocrine factors, measuring fecundity, gonado- and hepato-somatic index (GSI and HSI), plasma vitellogenin, and two indicators of oxidative stress catalase (CAT) and glutathione peroxidase (GSH-Px) concentration on days 2, 10, and 21 of the exposure. Additionally, we undertook a histopathological evaluation of fish gonads and livers at the end of the exposure. Copper significantly affected fecundity at both 20 and 60 $\mu\text{g L}^{-1}$ Cu with reductions in both spawning frequency and number of eggs per spawning event observed. Corresponding to these effects, both CAT and GSH-Px concentrations increased in a dose- and time-dependent manner in both the gonads and liver. Concentrations peaked on day 10 and then declined by day 21, suggesting antioxidant concentrations could not be sustained throughout the entire exposure. Plasma vitellogenin concentrations, GSI, and HSI were not affected by Cu exposure at any concentration. Histological analysis revealed increased hepatic bile duct hyperplasia in male fish. In females, there was a reduction in post-ovulatory follicles in the 20 and 60 $\mu\text{g L}^{-1}$ Cu treatments, an increased prevalence of oocyte atresia in the 20 but not 60 $\mu\text{g L}^{-1}$ treatment, but no monotonic effect on ovarian developmental stage. Overall, we conclude that folliculogenesis may have been disrupted in the higher Cu treatments resulting in reduced fecundity. Importantly, the lack of changes in GSI and HSI combined with no change in plasma vitellogenin concentrations suggests that the reduction in fecundity is not due to endocrine-mediated effects. The significant increase in CAT and GSH-Px concentrations in the gonads indicates that these tissues were under considerable oxidative stress, which may have contributed to the reduced fecundity. However, histopathology results were somewhat ambiguous and did not provide consistent support for this mechanism leaving uncertainty on the exact adverse outcome pathway that leads to reduced fecundity.

1.12.P-Tu154 Developmental and Reproductive Toxicity of Bisphenol AP in the Great Pond Snail *Lymnaea stagnalis*

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As a result of increasing regulations on bisphenol A (BPA), classified as endocrine-disrupting chemical (EDC), BPA alternatives are entering the market. Concerns are rising as contaminations of the environment have already been reported. In this study, we want to use new approach methodologies (NAMs) to assess the risk of BPA alternatives. We investigated the effects of Bisphenol AP (BPAP) on both the embryonic development and the reproduction of the great pond snail *L. stagnalis*, using endpoints described in the literature, as well as the OECD test guideline 243. Briefly, embryos were exposed 18 days to 0 (control), 31.25, 62.5, 125, 250, 500, and 1,000 $\mu\text{g/L}$ of BPAP. For the reproduction test, adults snails were exposed in semi static conditions to five BPAP concentrations, from 31.25 to 500 $\mu\text{g/L}$, with a 2 factor. The BPAP concentration was checked in the exposure media for both tests using a UHPLC-3Q-MS/MS. Apart from the embryonic rotation, all endpoints were significantly affected for at least two exposure groups in both test. For the embryos, the heartbeat was significantly lower than the controls for all concentration groups, and their size at day 9 of the embryos exposed to 500 and 1,000 $\mu\text{g BPAP/L}$ was respectively 26.8 and 65.6 % smaller than the controls. At the end of the test, only 50 % of the embryos exposed to BPAP at 500 $\mu\text{g/L}$ had hatched, and 100 % of those exposed to 1,000 $\mu\text{g/L}$ had died. For the reproduction test, the lowest BPAP concentration (31.25 $\mu\text{g/L}$) was enough to reduce the number of laid egg masses by 59.43 % compared to the controls. We determined a BPAP 28d-EC50 for the reproduction of *L. stagnalis* at 38.7 $\mu\text{g/L}$, and an 28d-LC50 at 550 $\mu\text{g/L}$. The BPAP EC50 and LC50 are lower than these of BPA and BPE for other invertebrate species. Our results demonstrate a high sensitivity of *L. stagnalis* to BPAP, showing the relevance of this organism in toxicological studies for chemical risk assessment. We also present concerning data about the safety of the use of BPAP as an alternative to BPA.

1.12.P-Tu155 Chronic Effects of Bisphenol A and Three Structural Analogues on Freshwater Snails and Amphipods

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Due to its diverse endocrine disrupting potential, the EU has identified bisphenol A (BPA) as a substance of very high concern, requiring it to be substituted with safer, more sustainable alternatives. Potential alternative bisphenols, also referred to as analogues, were developed to replace BPA and are currently being implemented increasingly into the plastics production. While the toxicity of BPA is well studied, (eco)toxicological effects of the bisphenol analogues are largely unknown. However, the similar molecular structure of the analogues suggests possible comparable toxicological effects. Recent studies on bisphenol

analogues have shown strong estrogenic as well as anti-androgenic effects in vitro with similar or sometimes even higher endocrine potential than the reference BPA. A more comprehensive ecotoxicological evaluation is necessary to properly assess the risk of the effects of bisphenol analogues on the aquatic environment. This study evaluates the four bisphenols BPA, BPF, BPS, and BPAF in reproduction tests with two aquatic model organisms. The freshwater snail *Potamopyrgus antipodarum* was selected as an estrogen-sensitive invertebrate and the freshwater amphipod *Hyalella azteca* as an estrogen-insensitive invertebrate. Recorded endpoints include the production of offspring, mortality, length and dry weight. Snails exposed to concentrations of bisphenols ranging from 1 to 100 µg/L showed a significant induction in the production of embryos in all tested concentrations. The number of neonates per surviving amphipod female was not significantly affected by bisphenol exposure. The mortality of the amphipods was impacted especially in the highest concentrations (2 mg/L) of the bisphenols BPA, BPF, and BPAF. This study shows that bisphenol analogues can impact invertebrate offspring production in estrogen-sensitive organisms in the same concentrations as bisphenol A. The embryo production of *P. antipodarum* was affected in concentrations as low as 1 µg/L, posing a serious risk for estrogen-sensitive organisms in freshwater systems.

1.12.P-Tu156 Effects of BPS on the Early Developmental Stage of Stickleback

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Bisphenol A (BPA) is an endocrine disruptor known to negatively impact aquatic organisms. Due to its toxicity and persistence, BPA has been restricted, leading to the introduction of substitutes like bisphenol S (BPS). However, the effects of these substitutes are still unclear. Endocrine disruptors affect sensitive developmental periods, potentially disrupting energy balance, which is crucial for survival, reproductive success, and population dynamics. This study investigates the effects of BPS exposure on the early developmental stages of the three-spined stickleback (*Gasterosteus aculeatus*), a model species sensitive to endocrine disruptors. Fertilized eggs (n = 1151) were randomly distributed in 10-liter tanks injected with BPS at concentrations of 0, 1, 10, or 100 µg/L. Exposure lasted for 38 days. Growth and mortality were monitored, with sample collection at 14 days post-fertilization (dpf), corresponding to yolk sac resorption, and at 38 dpf, marking the end of exposure when individuals were fully developed adults. Samples will be used to evaluate sex ratios, lipid and glycogen levels between groups. Fish in the control group attained a higher average size compared to those exposed to BPS. Increasing BPS concentrations were associated with a progressive decrease in growth. At day 8, the hatching rate in the control group was 54%, while it was approximately 8% for all BPS concentrations (1, 10, 100 µg/L). By day 9, the hatching rate reached 93% for the control group, and 84%, 79%, and 74% for 1, 10, and 100 µg/L, respectively. By day 10, the hatching rate exceeded 95% for all concentrations. These results indicate that BPS exposure delays fish hatching. Sex ratio, lipid and glycogen levels analyses will be conducted to further assess on the physiology. The results will be presented during the congress. This study shows significant sub-lethal effects of BPS exposure on stickleback growth and development, indicating potential risks of using BPS as a BPA substitute. These findings highlight physiological disruptions and support the need for refined risk assessments and stricter regulatory guidelines. This work was carried out in the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC) and has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement No 101057014. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the Health and Digital Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

1.12.P-Tu157 Assessing Safety of BPA Alternatives: Impacts on Thyroid hormone System (THS)-and Developmental Neurotoxicity (DNT) Sensitive Endpoints in Zebrafish (*Danio rerio*)

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We investigate if Bisphenol A (BPA)-alternatives like BPE, BPAP, and BPS-MAE affect the thyroid hormone system (THS)-and developmental neurotoxicity (DNT)-sensitive endpoints in zebrafish (*Danio rerio*). Adverse effects on THS-sensitive endpoints such as a decrease in retinal pigmentary epithelium (RPE) have earlier been reported from BPA in zebrafish. It has also been documented that a decrease in thyroxine (T4) and 3,5,3'-triiodothyronine (T3) in early-life zebrafish larvae can be connected to altered eye development and specifically the retinal pigmentary epithelium (RPE) and delayed inflation of the posterior swimbladder chamber in zebrafish larvae. Additionally, research shows that T4 and T3 play a crucial role in development of the brain in vertebrates. We want to investigate if THS and/or DNT-sensitive endpoints adversely affect the swimming performance, which can be considered an adverse endpoint with population relevance. We demonstrated that some BPA-alternatives like BPE, have the

potential to be endocrine disrupters. We found adverse effects on thyroid hormone system-sensitive endpoints in zebrafish embryos due to exposure to BPE. We want to present if the same tendency applies to other BPA alternatives like BPAP and BPS-MAE. Additionally, we want to present the possible effects of BPE, BPAP, and BPS-MAE on developmental neurotoxicity sensitive endpoints, such as, structural changes in the brain. This partnership has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement No 101057014

1.12.P-Tu158 New Approaches in the Evaluation of the Thyroid Disruptive Potential of BPA Analogues

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Bisphenol A (BPA; 2,2-bis(4-hydroxyphenyl)propane) is a synthetic chemical employed in the plastic industry to harden plastics, as an antibacterial agent, and in anti-corrosion coatings. It is used in the elaboration of polycarbonate plastic, a common material found in food and water storage containers, in thermal paper and epoxy resins. In Europe, BPA has been classified as toxic for reproduction and as a candidate substance of very high concern (SVHC) based on its endocrine disruptive potential. Although the European Commission and other governments have proposed its ban in food contact materials and other uses, industries are replacing BPA with other bisphenol analogues with structural similarities such as BPS, BPF, BPE, BPP, BPZ, TBBPA, among others. Some of these substances have been already found in human and environmental matrices but their health effects and mechanisms of action are still largely unknown, with some evidence pointing towards endocrine disruption properties. Therefore, our main objective, framed within work package 5 of PARC (Partnership for the Assessment of Risks from Chemicals), was to evaluate the potential of the above-mentioned BPA analogues as thyroid hormone disruptors using recently developed in vitro test methods. We focused on the effects on iodide and thyroid hormone (TH) transport by their respective transporters NIS and MCT8, as well as their potential to disrupt TH-related gene expression in thyroid cellular models. Our results show that BPA and its analogues altered TH-related gene expression, but effect direction and degree varied depending on the analogue studied, some of which were even more detrimental than BPA. TH and Iodide transport were also affected with similar IC50 results for BPA and its analogues. Our results highlight the importance of new methodologies to further assess the endocrine disruption capabilities of bisphenol substances, especially for early life stages, as well as highlighting the urgency to include these substances in the SVHC candidate list. Financed by: MCIN PID2021-125948OB-I00/AEI/10.13039/501100011033 and PARC project (GA 101057014).

1.12.P-Tu159 CRISPR/Cas9 Makes its Way into Ecotoxicology – A Tale of Estrogenic Antagonism *Gustavo Guerrero-Limon¹ and Marc Muller², (1)Vitis Regulatory, Belgium, (2)University of Liege, Belgium*

To mimic the effects of endocrine disrupting chemicals in zebra fish, we deployed a novel method using the gene editing tool (CRISPR/Cas9) to deactivate the function of specific oestrogen receptors. Endocrine disrupting chemicals are a pressing issue that have been in the spotlight for some time now. Their modulating action on endocrine signalling pathways makes them a particularly interesting topic of research within the field of ecotoxicology. Traditionally, endocrine disrupting properties are studied using exposure assays to suspected chemicals. In this research editing tools have been applied to directly assess the function of specific genes. Among these, the CRISPR/Cas9 method has accelerated progress across many disciplines in biology, helping the elucidation of specific interactions by knocking in or out targeted genes. This versatile tool was applied to directly inactivate three oestrogen receptors in zebrafish (*Danio rerio*) aimed at mimicking the antagonistic properties of endocrine disruptors. Then, using a panel of biological tests that is commonly applied to assess the overall development of zebrafish larvae, we investigated the antagonistic effects that exposure to endocrine disrupting chemicals would have. We found that the absence of individual functional oestrogen receptors (*Esr1*, *Esr2b*, or *Gper1*) affect behaviour, heart rate and development. Each mutant line was viable and could be grown to adulthood, the larvae tended to be morphologically grossly normal. However, a substantial fraction (70%) of the *esr1* mutants presented severe craniofacial deformations, while the remaining 30% of *esr1* mutants also exhibited changes in behaviour. *esr2b* mutants had significantly increased heart rate and significant impacts on craniofacial morphometrics. Finally, mutation of *gper1* affected behaviour, smaller size (decreased standard length), and decreased bone mineralization as assessed in the opercle. Although the exact molecular mechanisms underlying these effects will require further investigations, we successfully

tested a novel way to assess antagonism with potential to help us understand better the effects of endocrine disrupting chemicals found in our environment. This research project received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie Innovative Training Network (ITN) program PROTECTED, Grant agreement No. 722634.

1.12.P-Tu160 QSAR in Silico Model for Predicting Thyroid Receptor Endocrine Disruption Potential

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Interest in the impact of endocrine-disrupting chemicals (EDCs) on human health and ecosystems is growing globally. Regulatory agencies have developed frameworks for EDC identification involving in vitro and in vivo testing of Estrogenic, Androgenic, Thyroid and Steroidogenesis (EATS) modalities. While certain E, A and S receptor assays are well-validated, thyroid receptor-specific assays are underdeveloped and lack comprehensive validation. Current regulatory methods rely on in vivo studies of reproductive and developmental toxicity which do not reveal specific interactions with thyroid receptor subtypes (? and ?). Such knowledge gaps are especially significant for petrochemicals and high-production-volume substances. To address these limitations, new approach methodologies (NAMs), including quantitative structure-activity relationship (QSAR) models, are essential for rapid, cost-effective predictions of chemical interactions with endocrine system targets, thus minimizing reliance on animal testing. This study introduces a novel QSAR model designed to predict thyroid receptor-mediated endocrine activity, underpinned by an innovative data curation methodology. The curated dataset was mainly sourced from EPA ToxCast, and refined through a semi-automated curation process guided by expert criteria, ensuring high data quality. The data splitting was performed using the Kennard-Stone algorithm to ensure structural diversity and balanced distribution across the training, validation, and test sets, while also considering the response to achieve better representativity of the target properties. A machine learning algorithm, namely a support vector machine (SVM) using circular molecular fingerprints, was trained and validated. The model demonstrated robust performance, with sensitivity, specificity and f1-score values of 92, 86 and 89% for the validation set, and 84%, 99%, and 87% for the external test set. A defined applicability domain further enhanced prediction reliability, meeting OECD principles for QSAR validation and ECHA regulatory standards. ? Our model's unique curation methodology improves data quality, facilitating more reliable predictions and filling critical gaps in thyroid receptor research. This QSAR tool efficiently identifies endocrine disruption potential, supporting regulatory and industrial assessments, allowing prioritisation of chemicals with lower EATS modality risk, and promoting faster hazard assessments while reducing animal testing.

1.12.P-Tu161 Molecular Modeling Framework to Predict Protein-Ligand Interactions to Detect Endocrine Disrupting Potential of Chemicals, Upgrade and Validation

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The global effort to identify substances with endocrine disrupting (ED) modalities currently benefits from various computational methods which stand as an alternative but also as a complementary tool to experimental studies. Molecular docking in contrast to other computational techniques requires a minimal amount of training data to establish predictive models. Under the limit of available atomic-scale protein structures, docking can rapidly investigate the possibility for interactions between a protein involved in ED pathways and small molecules of interest. Our team actively develops a framework for acquisition and analysis of molecular docking results. The tool described in this work relies on trustworthy third-party models for the heavy lifting of docking related computations, while automation on preparation of inputs, raw data analysis and cohesion between different external tools is done by in-house programs. Our docking framework differs from other tools by exploiting structural ensembles to generate docking results, moreover, predictions are cast upon statistical assessment of the similarities between ensembles describing the reference and the tested data. In a recent communication we reported that despite the inherent low specificity due to the lack of crucial analysis stages, the framework correctly classified 223 out of 238 in vitro positive molecules. Furthermore, 141 experimental positives were ranked better than decoys (among the 185 of decoys included in the dataset), hence an enrichment factor between 0.56 to 0.73 for six EATS modality targets (Estrogen alpha and beta, Androgen, Thyroid alpha and beta, Progesterone receptors). Here, we present results for a small-batch screening study (external dataset including experimental positives and negatives) on six EATS targets. These results were obtained with the recently updated version of the framework that was developed to correct for the low specificity of the analysis module that was observed previously. Despite the higher computational cost in comparison to trained models this work demonstrates that molecular docking is affordable even for screening studies and is a valuable tool providing mechanistic evidence for prioritizing compounds with respect to their putative ED potential.

1.12.P-Tu162 In Silico Tools for Endocrine Disruption Assessment: A Focus on Human Transthyretin Disruptors

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Endocrine Disrupting Chemicals (EDCs) are a structurally heterogeneous group of substances of widespread use and application, whose identification and assessment has become priority due to their ability to negatively affect the endocrine system in living organisms. In this context, the use of in silico approaches, such as Quantitative Structure-Activity Relationships (QSARs), is suggested to fill data gaps and to support the identification of substances with endocrine activity. This work aims to develop new QSAR models for the prediction of the binding affinity of exogenous chemicals with human transthyretin (hTTR), identified as a relevant molecular initiating event leading to thyroid hormone (TH) system dysfunctions. The modelled endpoint was the logarithm of the Relative competitive Potency (RP), which reflects the ability of a compound to displace the TH thyroxine (T4) from the hTTR. The here proposed models were developed using three new datasets that include, to our knowledge, all the currently available experimental values of RP published in the literature and measured with three different in vitro assays: the radiolabeled [125I]-T4 binding assay (RLBA), the 8-anilino-1-naphthalenesulfonic acid (ANSA) based binding assay, and the fluorescence conjugate isothiocyanate (FITC)-T4 based binding assay. Each dataset is composed of data measured by the same in vitro assay. Theoretical molecular descriptors were generated from harmonized SMILES (Simplified Molecular Input Line Entry System) and were used as variables in QSAR modeling. QSARs development followed the OECD guidelines, in order to guarantee for the internal statistical reliability and the external predictivity of the models when applied to new chemicals. The mechanistic interpretation of the selected molecular descriptors provided insights on the structural features that promote the displacement of T4 from hTTR. Due to the presence of different chemical structures in the three datasets, each QSAR was applied through the QSAR-ME Profiler software to fill missing values in the other two datasets. Compounds with reliable predictions were ranked by PCA analysis according to their experimental or predicted RPs, to discriminate stronger and weaker hTTR binders. The proposed QSARs can be applied to support the identification of chemicals with potential TH system-disrupting activity, which could represent a severe threat for human health and wildlife.

1.13 Challenges and Innovations in Assessing Chemicals and Mixtures with Difficult-to-Test Properties for Environmental Risk

1.13.T-01 Tripartite Perspectives on Optimizing UVCB Testing: Balancing Whole Substance and Representative Constituent Approaches

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Complex substances such as multi-constituent substances and UVCBs usually result from either industrial processing, natural substances, or chemical reactions, and make up approximately 20-25% of chemicals registered globally (e.g., under TSCA or REACH). Because of the variable and complex nature of source materials, and variability inherent to production processes, these substances can contain many, sometimes uncharacterized, constituents whose concentrations may vary between batches. To identify and advance the various challenges associated with UVCB testing and assessment, the Health and Environmental Sciences Institute (HESI) organized an international workshop Exploring the complexities of UVCB testing and risk assessment that took place on September 18th and 19th, 2023, in Reykjavik, Iceland. The 25 workshop participants represented academia, governments and regulatory agencies, as well as, the private sector, and originated from Belgium, Canada, Denmark, France, Germany, the Netherlands, Norway, the United Kingdom, and the United States of America. The HESI UVCB workshop aimed at initiating multi-sectoral, tripartite discussions on the advantages and disadvantages of whole substance vs. representative constituent testing and assessment, and how to best combine both approaches. Workshop attendees also identified further research needs, and attempted to establish potential consensus solutions for environmental risk assessment. We will present the insight from the workshop, which contributed to reinforcing the exposure-centric tiered approach previously developed by the Committee for the environmental risk assessment of UVCBs and multi-constituent substances. The case-study based discussions held during the workshop highlighted critical questions that ultimately helped provide some

guidance to strategically test different aspects of whole UVCB substances and their representative constituents. Altogether the discussions and case-studies from the workshop generated information that will serve as a foundation to robust risk assessments.

1.13.T-02 UVCB Biodegradation Testing Challenges and Solutions

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Persistence assessment of chemicals is conducted through a tiered approach for biodegradation testing from ready biodegradability screening tests to simulation biodegradation tests. However, these standard tests are not applicable for complex mixtures such as UVCBs (substance of Unknown or Variable composition, Complex reaction products or Biological Materials). The main testing challenge is the complex nature of UVCBs, since standard tests use non-specific measuring parameters that cannot reveal the degradation of individual UVCB constituents. Biodegradable major constituents could then mask persistent minor constituents. The aim of our work was therefore to develop biodegradation tests for UVCBs that combine whole substance testing with constituent specific analysis. This presentation will cover the development and dimensioning of a ready biodegradability test and a simulation biodegradation test for UVCBs. The dimensioning targeted also the challenges posed by volatile and hydrophobic constituents, present in many UVCBs, and prone to losses during testing. The developed methods were demonstrated with black pepper and lavender essential oil UVCBs. The simulation biodegradation tests were conducted at low test concentrations and constituent specific primary biodegradation of unknown constituents was obtained through Arrow SPME GC-MS-scan. The ready biodegradability test was conducted at a higher test concentration confirmed to be below inhibitory levels. It combined whole substance mineralization through contact less oxygen measurements with the primary degradation of UVCB constituents through SPME GC-MS-scan analysis. The experiments showed fast degradation of essential oil constituents. The study demonstrates how biodegradation at different test tiers can be determined for UVCBs with a careful design of the experiments.

1.13.T-03 Ecotoxicity Testing of Cationic Surfactant Benzalkoniums (BACs) Using a Novel Passive Dosing Method

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In aquatic toxicity tests, controlling and describing the exposure conditions is challenging when the substance is susceptible to absorptive loss within the test system. To address those issues, this study introduced a new passive dosing method in the acute immobilization test on *Daphnia magna* (OECD TG No.202) of cationic surfactants benzalkoniums (BACs) with the alkyl chain length of 14-18 carbons. Thirteen materials were tested for their sorption capacity in choosing a suitable passive dosing material. Series of loading and desorption experiments were conducted to examine the stabilization of the exposure concentration (C_w) via equilibrium partitioning. Consequently, the 48h-acute ecotoxicity tests of benzalkoniums on *D. magna* using passive dosing and conventional solvent spiking methods were compared in terms of C_w stability and toxicity results. The results showed that poly(ethersulfone) (PES) membrane appeared to be an optimal passive dosing reservoir (i.e., $\log K_{PESw} \sim 4$), with which equilibrium desorption was reached within 24 h under gentle shaking and the C_w remained constant afterward. In the acute ecotoxicity, the C_w stayed unchanged during the testing period in both the dosing methods. However, in the solvent spiking test, the C_w was lower than the nominal concentration (C_{nom}) for C12-C18-BACs, particularly at low exposure concentrations with a difference of 20-60 % compared to C_{nom} . The observation in the toxicity of BACs revealed that the solvent spiking tests displayed the U-shape trend of the median effect concentration (EC_{50}), i.e., decreasing from C6-BAC to C14-BAC and increasing from C14 to C18-BAC. In contrast, EC_{50} values of BACs from C14 to C18 measured by the passive dosing method were similar or slightly decreasing, implying higher toxicity of C16 and C18-BACs than those performed by the solvent spiking method. The toxicity tests indicated the 48h- EC_{50} values of the long-chain BACs to *D. magna* were at single $\mu\text{g/L}$ range. The findings in this study suggest the potential of applying this innovative passive dosing approach in the extension to other aquatic organisms in both acute and chronic toxicity tests of BACs or other cationic surfactants under relevant environment conditions, which should be useful support for risk assessment of cationic surfactants in the aquatic environment.

1.13.T-04 The Influence of pH on the Toxicity of Ionizable Agrochemicals to Freshwater Invertebrates

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A wide range of environmentally concerning chemicals belong to the group of ionizable organic chemicals (IOCs). Among agrochemicals, up to one-third are ionizable. IOCs can exist in aquatic environments in multiple forms, changing their form based on pH variations determined by their acid dissociation constants (pKa). The neutral form of IOCs has a higher potential to reach target sites within organisms and is often observed to be more toxic. Therefore, it is essential to understand the pH-dependent behaviour of IOCs to accurately assess their environmental impact. However, directly assessing the toxicity of IOCs at different pH levels is not easy, as pH can also impact the functioning of biological systems in addition to influencing the dissociation of IOCs. To disentangle these issues, we raised several research questions: (1) Does pH alone cause adverse effects on aquatic species? (2) Do non-ionizable chemicals exhibit pH-dependent toxicity? (3) Do ionizable chemicals show varying toxicity across pH levels, and what theoretical framework explains this? To answer these questions, we conducted three tests. Test 1 assessed the mortality of five aquatic species across pH levels 6.5, 7.5 and 8.5, showing less than 20% mortality in four species and 33% mortality in *Gammarus pulex* at pH 8.5. Test 2 evaluated the toxicity of the non-ionizable compound flupyradifurone on *Cloeon dipterum* and *Hyalella azteca* at the same three pH levels, showing no pH-dependent toxicity. Test 3 investigated the toxicity of the ionizable fungicide fluazinam (weak acid, pKa 7.34) across the three pH levels, showing that pH influences both chemical degradation and toxicity. Preliminary results showed that (1) the chemical degraded more rapidly at lower pH levels; (2) the EC₅₀ is five times lower at pH 6.5 compared to pH 8.5 for *H. azteca*, (3) a strong linear regression supported the ionizable toxicity theory that both the neutral and ionized forms are active and act additively in toxicity. Our study systematically addresses concerns regarding ionizable chemicals by demonstrating that the influence of pH on these compounds is driven by their chemical dissociation rather than direct pH effects on biological systems. As water pH levels in European surface waters can easily vary from 6 to 9, the finding that fluazinam's EC₅₀ is five times lower at 6.5 than at pH 8.5 can have implications for its ecological risk assessment under worst-case pH scenarios.

1.13.T-05 From Theory to Practice: Tackling Challenges and Innovations in Assessing Risks from PFAS and Tyre Wear Particles

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The SOS-ZEROPOL2030 project supports the EU's ambition of achieving "Zero Pollution" in its seas. It focuses on two challenging pollutants: per- and polyfluoroalkyl substances (PFAS) and tyre wear particles (TWP). These case studies, selected for their complexity and data gaps, stress-test a scenario-based risk assessment framework addressing varying levels of data availability: Scenario 1 assumes sufficient exposure and hazard data, enabling robust assessments; Scenario 2a/2b involves data gaps in either exposure or hazard data, requiring models to fill gaps with reduced robustness; Scenario 3 relies entirely on models due to insufficient data, introducing significant uncertainties. The framework identifies data gaps and shows how modelling can partially address these, contributing to the SOS-ZEROPOL2030 roadmap. PFAS exposure data is abundant in northern European seas but limited in the Mediterranean and Black Sea. While sufficient toxicity data exists for legacy PFAS like PFOS and PFOA, data on novel PFAS are limited. Assessments show over 25% of sampling stations in some regions exceed toxicity thresholds for legacy PFAS, while fewer exceed thresholds for novel compounds. Challenges include inconsistent monitoring, limited data coverage, and analytical constraints. Recommendations include expanding monitoring to cover novel PFAS, improving analytical methods, and conducting long-term data collection. TWP assessments examined TWPs, tyre wear leachates (TWLs), and tyre wear chemicals (TWCs). Insufficient empirical data necessitates reliance on models. Findings suggest TWP emissions in water rarely exceed thresholds but highlight concerns about sediment accumulation and TWL/TWC toxicity. TWCs, as complex mixtures, complicate measurement and attribution in risk assessments. Recommendations include developing methods for TWP quantification and TWC identification, generating toxicity data on sublethal and chronic effects, and incorporating TWCs into monitoring programmes. SOS-ZEROPOL2030 highlights the need for enhanced data collection, innovative modelling, and coordinated policies. Its framework provides pathways for sustainable solutions aligned with the EU's zero-pollution vision, such as an EU-wide ban on non-essential PFAS and technological solutions to reduce emissions of TWPs in urban hotspots, enhanced monitoring, and the promotion of PFAS-free alternatives or alternative transport mechanisms. SOS-ZEROPOL2030 is funded by the European Union's Horizon Europe programme under grant agreement No. 101060213.

1.13.P Challenges and Innovations in Assessing Chemicals and Mixtures with Difficult-to-Test Properties for Environmental Risk

1.13.P-Th020 What Drives Alga Toxicity of Primary Fatty Amines?

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Primary fatty amines (PFAs) are high production chemicals bearing a set of properties which include them in the category of difficult-to-test-substances as defined in the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD 23). These substances can be biodegradable, multi-constituents/UVCBs as well as surface-active, adsorbing and ionic. In the range of environmental relevant pH-values PFAs are completely protonated (cationic surfactants) making it difficult to maintain stable concentrations in environmental toxicity and fate studies. This can be due to formation of micelles and dispersions and in addition due to adsorption processes to test vessels or negatively charged biological materials. Examples for this kind of material are algal cells, which are present in alga toxicity and chronic daphnia studies. Generally proposed adaptations of the test design of such studies include the usage of truly dissolved concentrations for the evaluation of the results. This concept is based on the idea that only the dissolved fraction of the test substance is bioavailable. However, in case of positively charged surfactants it is highly questionable if this represents the truth: Adsorption to the test organism most probably will lead to an increase of the local concentration rather than to a decrease as the decreasing dissolved fraction in the water phase suggests. To investigate the real distribution of PFAs in the test system a green alga study on growth inhibition with a radiolabeled primary fatty amine is performed. The truly dissolved fraction, as well as the amount of adsorbed substance to surfaces of different textures, are analytically determined. Based on the results the suitability of different evaluation schemes will be discussed.

1.13.P-Th021 Method Development and Analytical Challenges for the Quantitation of Complex Industrial Chemicals in Support of Environmental Fate and Ecotoxicology Studies

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Due to the evolving regulatory landscape for registration under REACH, environmental studies that were typically performed for crop protection chemicals are moving towards assessing a broader array of complex chemicals, including industrial chemicals, naturals, botanicals and chemicals of Unknown or Variable Composition, Complex Reaction Products or Biological Materials (UVCBs). The registration of these substances introduces unique challenges due to the nature of the chemicals themselves, ranging from technical constraints in conducting regulatory fate studies to the feasibility of radiolabelling and the analytical limitations encountered in non-radiolabelled ("cold") analysis. The nature of these substances also presents numerous challenges when also providing support for other study types, such as ecotoxicology studies, where difficulties in achieving the required levels of sensitivity to confirm application of the dose to the test media are frequently faced. Many industrial chemicals present numerous analytical challenges, due to a variety of issues associated with their often varied and complex composition. To develop suitable analytical methods, there are a number of considerations that need to be addressed. For example, determining the nature of the analyte is particularly important, as a single component is more straight forward to quantify as the properties of the molecule can be considered when selecting and optimizing a suitable quantitation technique. However, a multiple component mixture (e.g. UVCB) consists of a range of components of differing chemical properties and abundance, often leading to a representative marker or even multiple markers being selected for quantitation. It is desirable to monitor as many marker components as possible to provide a more thorough representation of the test material. There are numerous challenges when developing analytical methods for these types of test items, such as selecting and optimizing appropriate analytical instrumentation for the analyte or selected marker components, developing suitable extraction and extract clean-up techniques, assessing the possibility of including degradation products for certain study types as well as achieving successful validation of the resulting method. This poster describes the considerations, challenges and approaches to developing and validating analytical methods for complex industrial chemicals in support of environmental fate and ecotoxicology studies.

1.13.P-Th022 Sorption of Cationic Surfactants by Clay Mineral and Organic Matter: Isotherm Measurements with Conventional Batch and New Passive Sampling Methods

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Cationic surfactants are used in a wide range of applications, including disinfectants in disease control and fabric softeners in laundry aftertreatment. Due to their generally high toxicity to aquatic organisms, research on their environmental fate and effects is needed. Soil and sediment particles are often negatively charged and can strongly adsorb cationic surfactants. In this study, adsorption isotherms of the cationic

surfactants benzalkoniums (BAC) and dialkyldimethylammoniums (DADMAC) were measured on clay mineral (kaolinite) and organic matter (peat) samples. In parallel to the conventional centrifugation method, the free dissolved concentration was measured by the passive sampling (PS) method using a solid-phase microextraction fiber to evaluate the validity of the PS method. Kaolinite was pretreated with 1M NaCl solution to exchange any bound cations with Na⁺. Peat was washed with diluted NaOH solution to remove excess acids. Suspensions of these samples were prepared using 15 mM NaCl solution at pH 7. Batch adsorption experiments were performed by adding the suspension, target substance, and polyacrylate (PA) fiber to 2 mL microtubes. After 1 night to 24 h of rotary shaking, the tubes were centrifuged, the supernatant, PA fiber, and kaolinite were collected and extracted, and cationic surfactant concentrations were determined by LC-MS analysis. The same experiment was performed without natural sorbent to determine the sorption isotherms on the PA fiber.

The adsorption isotherms were all nonlinear (Freundlich exponent 0.5-0.7), although no clear surface saturation at high concentrations was observed for either material. The longer the chain length, the stronger the adsorption, but the increment of the adsorption coefficient per C was not constant. The results of adsorption experiments using the conventional centrifugal separation method and the PS method were comparable for long-chain BACs on kaolinite. However, different results were obtained for short-chain BACs on kaolinite and peat when investigated by the centrifuge and PS methods. It is likely that the fouling of the PA fiber with the natural sorbent affected the amount extracted for short-chain BACs. Based on the results, we conclude that the adsorption coefficients and isotherms of cationic surfactants can be measured by the PS method using PA fibers when the adsorption coefficient is PA >> natural sorbent and the effect of fouling is insignificant. This study was funded by JSPS KAKENHI JP22H03765.

1.13.P-Th023 Chemical Mapping of Natural Constituents through the Persistence, Bioaccumulation, Mobility and Toxicity (PBMT) Prism: Qualitative Analysis as a Precursor to Literature Data

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Ingredients from natural sources, in particular essential oils, are widely formulated in consumer products such as cosmetics, perfumery, and household products. Understanding the environmental and ecotoxicological impact of those Natural Complex Substances (NCS) has become a priority, notably with the implementation of the More than One Constituent Substances (MOCS) concept under the recent update of the EU Classification, Labelling and Package Regulation (EC No 1271/2008). However, the complex and variable composition of NCS presents a unique challenge for the assessment of their environmental impact. We will make use of the information gathered on over 1,000 natural constituents, as to their Persistence, Bioaccumulation, Mobility and Toxicity potential and statistical analyses that will help understand and predict the fate and effects of natural constituents. The first part of the study presents an innovative method of Hierarchical Clustering on Principal Components (HCPC), based on chemical families. The HCPC approach is particularly well-suited to the classification of complex substances, as it allows for the identification of distinct chemical families based on both structural similarities and environmental properties. This method clusters molecular fragments (qualitative data) and 2D descriptors (quantitative data), and is enriched by unsupervised statistical analysis methods such as Partial Least Squares (PLS) regression and sparse PLS. These advanced techniques enable us to uncover patterns in the molecular structures of natural constituents and establish correlations with key environmental parameters, such as persistence, bioaccumulation, mobility, and toxicity. In the second phase, a visualization based on regulatory thresholds for PBT/PMT is used to categorize clusters of components according to CLP criteria. This study, by focusing on molecular structure, it enables the qualitative prediction of the environmental impact of substances. From the clusters created and the descriptors and fragments characterizing these groups, we will identify key molecular descriptors critical for the assessment of natural constituents. These findings are crucial for advancing the understanding of the environmental fate of these substances and the applicability of the MOCS concept to substances of botanical origin, for which there is a 5-year derogation.

1.13.P-Th024 Application of GC×GC methodology to Assess the Bioaccumulation Potential of Hydrocarbon UVCBs in Fish

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The assessment of chemical bioaccumulation in fish is an important component of global chemical assessment and regulatory programs. Bioaccumulation assessments for petroleum substances remain

difficult because (i) they are comprised of complex and variable combinations of hydrocarbons (i.e., UVCBs); and (ii) there are limited test data for the thousands of possible hydrocarbon constituents in petroleum substances, due to limited commercial availability of the individual constituents and/or their incompatibility with standard test systems. Furthermore, standardized tests tend to be optimized for single constituents and may not function well for complex substances, as individual components within the substance may differ widely in their partitioning behaviour and biological fate. In the present study we characterize the dietary accumulation of a petroleum substance (i.e., middle distillate oil) in rainbow trout using an OECD 305 dietary bioaccumulation test design. Here we use two-dimensional gas chromatography (GC×GC) analytical and data integration methods to characterize the uptake and elimination of constituents in fish. This whole-substance-with-peak-tracking test design has been successfully applied in seawater biodegradation tests. However, the biological samples collected in bioaccumulation tests present additional challenges for adequate peak separation and identification in GC×GC analyses. In a previous pilot study, fish food and tissue matrices interfered with hydrocarbon peak separation and identification. To remove residual lipids and polyenes, we implemented alkaline digestion and epoxidation steps which greatly reduced analyte interference and carryover between chromatogram runs. Addition of an HPLC pre-fractionation step to separate saturated and aromatic hydrocarbon fractions also greatly reduced peak overlap and interference. We have now applied these improvements to evaluate bioaccumulation and biotransformation behaviour of hydrocarbon UVCBs in fish using a whole-substance-with-peak-tracking test design.

1.13.P-Th025 Evaluation of the Environmental Safety of Basil Extract (*Ocimum basilicum*) in Aquaculture Feeds

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Plant extracts (PEs) and essential oils (EOs) show potential as natural antimicrobials. These compounds are volatile, typically colorless, lipid-soluble mixtures composed mainly of terpenes and terpenoids. In veterinary medicine, especially for pets and livestock, PEs and EOs may serve as promising alternatives to synthetic antimicrobials, potentially useful against infections and improve production quality, without leaving the residues commonly associated with synthetic drugs in food products. Supercritical fluid extraction (SFE), often using solvents like carbon dioxide (CO₂), is the primary method for obtaining PEs and EOs. Although EOs and PEs are increasingly used in livestock, their environmental impact remains under-researched, likely due to the assumption that plant-based compounds are naturally safe. In this study, basil supercritical fluid extract (F1-BEO) was chosen, as basil (*Ocimum basilicum*) has shown antioxidant, antimicrobial, insecticidal, nematocidal, and fungistatic properties, especially relevant in aquaculture. Ecotoxicity tests were conducted under controlled conditions, using fish feed supplemented with 3% w/w of F1-BEO (substance A), pure F1-BEO extract (substance B), and fish feed without F1-BEO (substance C). These were tested across three model species from different trophic levels (bacteria, primary producers, and primary consumers) representing both freshwater (*Aliivibrio fischeri*, *Raphidocelis subcapitata*, *Daphnia magna*) and marine (*A. fischeri*, *Phaeodactylum tricornutum*, *Paracentrotus lividus*) ecosystems. The results showed a reduction in bioluminescence of *A. fischeri* at tested concentrations only for substance A. Dissolving fish feed in algal media promoted microalgal growth without any observed toxic effect. However, primary consumers in both freshwater and marine environments exhibited acute toxicity across all substances tested. Notably, *P. lividus* displayed higher embryotoxicity with substances A (EC₅₀ 1.80 mg/L) and C (EC₅₀ 4.6 mg/L) compared to substance B (EC₅₀ 7.10 mg/L), implying toxicity linked to feed dissolution. In contrast, for *D. magna*, substance B exhibited greater toxicity (EC₅₀ 0.34 mg/L) than substances A (EC₅₀ 3.98 mg/L) and C (EC₅₀ 5.50 mg/L). These substances may pose potential toxicity risks within aquatic ecosystems, particularly for primary consumers. Further research on PEs and EOs is essential to understand their environmental impact and behavior in aquatic environments more comprehensively. This study was funded by Resil Trout Project (23E03).

1.13.P-Th026 Building a Pilot Database of UVCB Chemical Characterization Information: Compiling Data on Substances of Unknown or Variable Composition, Complex Reaction Products, and Biological Materials (UVCBs)

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Substances of unknown or variable composition, complex reaction products, and biological materials (UVCBs) represent 20-25% of the chemicals registered globally and pose unique challenges for risk evaluation. To help address these challenges, a tripartite group within the Health and Environmental Sciences Institute (HESI) has been developing a tiered, exposure-based UVCB framework to guide prioritization and risk assessment of UVCBs. The initial step of the proposed approach (Tier 0) focuses on basic characterization to flag potential hazards, persistence, or bioaccumulation. However, this evaluation is highly dependent on the availability of Tier 0 data, including rudimentary exposure, compositional, and ecotoxicity data. Despite the prevalence of UVCB substances in the chemical space, the publicly accessible data available for these substances is often limited. Moreover, substance characterization in these databases is incomplete or incorrect leading to issues associated with linking data to a specific UVCB substance. Altogether, this underscores the need for a fit-for-purpose, publicly accessible UVCB database. A subgroup of the HESI UVCB committee is developing a pilot database of Tier 0 information for UVCBs to assess feasibility and develop efficient data-collation methodologies. Data collected includes substance identifiers, structural descriptors, registration data, physicochemical properties, use and process information, and ecotoxicity data. These data are being collated for both whole substances and representative constituents from online databases, registration dossiers, and scientific literature. The methods and challenges encountered during this effort have been documented to allow for reproducibility and aid in identifying potential improvements in future efforts to expand the database (e.g., steps to automate the data mining processes). This pilot demonstrates the feasibility of the project and highlights the challenges associated with developing a UVCB database. It also empowers registrants, risk assessors, and regulators to apply the UVCB framework more effectively by providing information that can be utilized for prioritization, risk assessment, and read across. Support from industrial and regulatory partners will be vital in facilitating data collection and the realization of the full potential of such a database. The views expressed in this article are those of the authors and do not necessarily represent the views or policies of the US EPA.

1.13.P-Th027 Graphene Grey Areas in Daphnia Ecotoxicity Exposures: Mixtures, Movement and Monitoring

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Graphene and silicon nanomaterials are widely used as dry lubricants for sporting equipment, boats, machines, engines etc. Aquatic ecotoxicity assessments with these materials is challenging as they are often hydrophobic due to the surface properties of the particles, in addition, they can often be applied in combination with mineral oil to act as a carrier for the particles, further increasing the hydrophobicity and changing the surface properties. The ecotoxicity of these advanced materials is important to understand, including any potential adaptation to the standard aquatic testing approach to take into consideration the physico-chemical properties of the materials and how they may change in different environments. *Daphnia magna* have widely been used for ecotoxicity testing of different materials, including chemical, advanced materials (including nanomaterials) and microplastics in isolation and in varying combinations. Due to the robust foundation of endpoints and research to date, *Daphnia* are an excellent model to explore aquatic risk in freshwater environments. A panel of commercially available materials were assessed; Graphene, Graphene + Silicon, Silicon, Graphene + oil, mineral oil. This included the characterisation of the particles using DLS and zeta potential in control medium and also in the testing medium (with the *Daphnia*) to determine how the materials may change during the test period. Acute toxicity testing was also undertaken with *Daphnia magna* (Bham 2) following the OECD 202 TG recommendations, before sublethal testing was undertaken looking at molecular markers such as increase in ROS in addition to changes in behaviour such as movement and swimming. Variations with experimental design and exposure order were trialled to determine if this had any significant impact on the outcomes of the tests, for example, adding the toxicant to the test vessel prior to the medium and *Daphnia* vs adding the toxicant last, and subsequently using different ages/development stages of the *Daphnia* for sublethal assays. Advanced materials are challenging to assess due to the surface properties, in this case hydrophobic particles, which prove difficult to evaluate effectively using the current protocols which are optimised for chemicals. Modification, such as exposure order, age of *Daphnia* etc in addition to physico-chemical characterisation can increase our understanding of the risk of AdMa in freshwater ecosystems. MACRAMÉ has received

1.13.P-Th028 Why are Commercial Surfactants Difficult to Test? Examination of the Challenges associated with Chronic Aquatic Testing

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Chronic aquatic toxicity testing can be very complex but is often a standard information requirement for the production, import, and sale of detergent surfactants in the EU. The OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD GD 23) identifies surface activity as a difficult test substance property. However, commercial surfactants also tend to have a variety of properties making them difficult to test. They can be poorly soluble, toxic at low concentrations, photodegradable, rapidly biodegradable, adsorbing, ionized, multi-constituent, and/or UVCB substances. These properties can make it difficult to choose appropriate application procedures, test designs, and analytical strategies, even with due consideration of OECD GD 23 recommendations. Altogether, this can introduce significant testing difficulties, resulting in potential test failure and/or generation of effect values which are not suitable for quantitative risk assessment or classification and labeling. Moreover, this can pose challenges associated with adequately meeting registration requirements or regulatory deadlines, and can introduce animal welfare issues (e.g., excessive use of vertebrate fish during testing). As such, there is a need to identify pragmatic and tangible strategies through which chronic aquatic test requirements for surfactants can be addressed and optimized. Therefore, a project was developed through ERASM (<https://www.erasm.org>) which leverages existing data and the expertise of industrial scientists and research organizations to address the issue of conducting chronic aquatic toxicity testing with surfactants. This project has compiled proprietary and publicly accessible chronic testing data on non-ionic (alcohol ethoxylates), anionic (alkyl ether sulfates), and cationic (e.g., alkyl etherdiamines) surfactants, identifying difficult test properties for substances within each surfactant group, methods employed, and the challenges faced during testing. Supplemented with literature-derived data, this dataset will be used to generate guidance (akin to OECD GD 23) that can be employed while conducting chronic aquatic toxicity tests with detergent surfactants. Overall, this will optimize chronic aquatic toxicity testing with surfactants, improving the likelihood of test success and utility of generated data while reducing the use of vertebrate test organisms.

1.13.P-Th029 Providing Structures for Unknown Constituents of Hydrocarbon UVCBs: In-Silico 'De Novo' Creation of Nonpolar Hydrocarbons

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Petroleum substances (PS) are archetypical UVCBs (Unknown or Variable composition, Complex reaction products, Biological materials), and can consist of a few hundred (e.g., solvent Naphtha) to potentially more than quinquagintillion (10¹⁸) unique hydrocarbon constituents (e.g., bitumen). Yet, the identity has been fully resolved through traditional chemical analysis for only for a few hundred of these constituents. Current methods for in-silico creation of chemical structures recombine structural moieties (e.g., backbones and side-groups) present in existing chemical databases. While such methods perform well in creating simple paraffins (alkanes) and aromatics, they fail to create the more complex and numerous structures: olefinic (alkene) and naphthenic (cyclo-alkane and -alkene) aliphatics and aromatics. In this poster, we discuss our newly developed KNIME workflow which generates all stable hydrocarbon isomers atom-by-atom, using only basic chemistry rules (e.g., tetravalency, hybridization, bond lengths and angles). Currently, in excess of 1 billion (10⁹) unique C₅ - C₁₇ stable nonpolar hydrocarbon isomers have been created, in which the olefinic and naphthenic structures largely outnumber the paraffinic and aromatic structures.

The availability of such an exhaustive database could dislodge some of the current analytical and regulatory limitations related to hydrocarbon UVCBs: chromatographic retention indices and mass spectra (MS) could be predicted for these structures, to improve peak identification in non-target chromatographic and MS data. Concurrently, suspect screening can verify the existence of those hypothetical constituents which are of potential environmental or health concern and for which further testing might be required.

1.13.P-Th030 Optimizing OECD 236 FET for Challenging Substances: Evaluation of a Closed-System Design for Reliable Aquatic Toxicity Testing

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To assess the intrinsic hazard of chemicals to aquatic organisms, standardized toxicological tests have to be performed according to established test guidelines. While conventional designs are suitable for soluble and stable substances, challenges arise with substances having complex physico-chemical properties, as highlighted in OECD 23 and ISO 14442 guidance documents on aquatic toxicity testing of difficult substances and mixtures. Measures have to be taken to ensure exposure concentrations to be as stable as possible, e.g. testing in closed systems in case of high volatility. However, such custom-tailored test designs bare the potential to negatively impact the sensitivity of test organisms, due to e.g. impaired gas exchange, thus potentially introducing bias compared to the conventional design. For OECD 236 FET studies polystyrene plates are commonly used. Limitations of this design are reached when it comes to testing of highly volatile or highly absorbing substances or polymers for which leaching of polymers from the plates pose an irritating source of background contamination. In any case, assuring that the modified test design does not alter the sensitivity of the organisms is a crucial aspect to warrant the reliability of data. We evaluate the usability of PTFE-capped glass vials as a closed system for the OECD 236 FET for maintaining stable substance concentrations without altering the sensitivity of test organisms compared to the conventional design. In addition, this alternative might facilitate testing of substances with other challenging properties like highly adhesive substances or polymers. To assess possible variation in sensitivity, multiple parallel OECD 236 FET studies will be conducted with embryos from the same spawnings in either the conventional or closed-system design exposed to the reference substance of the guideline (3,4-Dichloroaniline). Further, the suitability of the closed-system design to maintain stable substance concentrations during the test period will be assessed with several highly volatile substances in comparison to the conventional design with and without daily water renewal. Additionally, the total organic carbon will be measured to assess its suitability for testing of polymers without background contamination.

1.13.P-Th031 Evaluating the Reliability and Relevance of (Eco)Toxicity Studies on Micro- and Nanoplastics: SciRAPplastic and PlasticCRED

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Pollution from micro- and nanoplastics is a growing concern for scientists, regulatory and political bodies, and the general public. Despite extensive scientific efforts to identify potential health and environmental effects, assessing the overall health risk based on current research remains challenging. This difficulty comes from several factors, including technical challenges and a lack of harmonized definitions and methodological practices in micro- and nanoplastic research. Furthermore, limited transparency and inadequate reporting undermine the comparability of published study results. Our work addresses these challenges by developing tools to systematically evaluate the reliability and relevance of (eco)toxicity studies on micro- and nanoplastic effects. We have reviewed and revised existing tools to match specific needs for micro- and nanoplastics research. More specifically, we have focused on two existing frameworks: Science in Risk Assessment and Policy (SciRAP) and Criteria for Evaluating and Reporting ecotoxicity Data (CRED). These include SciRAP for in vivo studies, SciRAPnano for in vitro studies, and nanoCRED for ecotoxicity studies. This work is further based on a comprehensive literature review of physicochemical properties, and critical technical and methodological concerns for research on micro- and nanoplastics. Furthermore, we developed reporting checklists and guidance, outlining critical information and technical or methodological aspects that should be considered and reported specifically in micro- and nanoplastic studies. The outcome of our work is three tools known as SciRAPplastic (in vivo and in vitro) and plasticCRED (ecotoxicity), tailored to evaluate the reliability and relevance of micro- and nanoplastic (eco)toxicity studies. These tools can be applied as part of a weight-of-evidence assessment to evaluate reliability and relevance of scientific studies for regulatory purpose. The reporting checklists and guidance aim to support researchers in reporting essential information to ensure their studies meet reliability and relevance standards in a regulatory context. This work contributes to the identification of (eco)toxicity studies that assess environmental and health effects of micro- and nanoplastics, which provide data that are both reliable and relevant to serve as scientific evidence for legislation. The authors thank the EU Commission for funding this project that is part of Plasticheal.

1.13.P-Th032 Investigating Intrinsic Toxicity of Rare Earths to Algae – How Far to Go When Ecological Relevance Is Limited?

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To fulfil the REACH information requirements in its registration dossiers, the Rare Earth Consortium has

performed algal growth inhibition studies (OECD 201) with a large part of the substances in their portfolio. Because of the strong interaction of rare earths with the phosphate in the test medium, standard tests do not yield information on the intrinsic toxicity of the rare earths, as all rare earths are precipitated when phosphate is in excess in the test medium (no exposure) whereas all phosphate is precipitated when the rare earths are in excess (no phosphate = no algal growth possible). Because reluctance was experienced of the CROs to look for an alternative phosphate source that would stay available to the algae during the test, no further testing was performed and a weight of evidence document summarising all data generated as well as existing literature data was written for inclusion in the dossiers. In this assessment, modelling with Visual Minteq confirmed dissolved rare earth and phosphate measurements that were made during the different experiments. It was concluded that the results of these standard tests are not useful for classification purposes or for PNEC/ERV derivation and that the secondary effect of phosphate deprivation is considering typical phosphate concentrations in the environment unlikely to occur at ecosystem level. Recently however, further attempts to investigate intrinsic toxicity of rare earths to algae have been made in several academic projects, trying to replace the inorganic phosphate source in the standard algal test medium by organically complexed forms, resistant to the complexation and precipitation with rare earths. Although these experiments confirm that rare earths show intrinsic toxicity to algae, robustness of the medium adjustments is to be further investigated. Identified issues as well as ecological relevance of the findings of these studies will be further discussed in this contribution.

1.13.P-Th033 Combination of Effect Directed Analysis and Pull-down Assay Coupled to Non-target Analysis for Identification of Endocrine Disruptors in Treated Wastewater

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While the quality of surface water has improved over recent decades, the presence of diverse micropollutants entering water bodies via treated wastewater (WW) remains a significant concern. These substances can induce adverse biological effects, posing risks to aquatic ecosystems and potentially impacting human health. Causative chemicals occur in highly complex mixtures, and often remain unidentified due to the absence of effective methods for their identification and analysis. This study employed an innovative strategy to improve the efficiency of the identification of endocrine disruptors in treated WW. Treated municipal WW was sampled using passive samplers that captured a broad spectrum of micropollutants over a 16-week period. The collected sample was analysed using two strategies aimed at simplifying sample composition and identifying bioactive compounds. The sample showed strong potency to inhibit binding of thyroid hormone thyroxine (T₄) to its plasma transport protein transthyretin (TTR). The observed bioactivity could not be explained by the compounds detected through targeted analysis. Consequently, effect-directed analysis (EDA) and pull-down assays (PDA), coupled with non-targeted analysis (NTA) based on HPLC-HRMS, were employed to separate and identify the causative chemicals. In the EDA, 2 levels of orthogonal fractionation were performed and 2 fractions with highest activity were subjected to NTA and further investigation. The direct NTA of these 2 fractions did not allow identification of suspected or novel TTR ligands due to the remaining high complexity. Both the full extract and the most active fractions were subjected to the PDA, utilizing highly specific protein-ligand interactions to separate the active ligands. These were addressed by NTA conducted in 2 independent laboratories and activity of several identified TTR ligands was confirmed in the TTR bioassay. The results demonstrated that combining EDA and PDA yielded the highest identification rate, as the fractionation steps facilitated the binding and detection of less potent and/or less abundant TTR ligands in pull-down assay. The 2 laboratories, each employing different NTA workflows, successfully identified several ligands, with each laboratory contributing additional ligands outside the overlap. Unlike EDA, the PDA showed sufficient reduction of chromatographic peaks in the highly complex environmental sample, making it an effective tool for identifying bioactive ligands.

1.13.P-Th034 Metrics for Structural Diversity in Chemical Mixtures

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Metrics are presented for the quantification of structural diversity in chemical mixtures. Structural diversity is quantified in terms of a calculated chemical distance between mixture components based on the Abraham solvent descriptors for mixture components. Three distance metrics for structural diversity are presented, one based on the maximum difference between Abraham descriptors for components (Max-Min), one in which the first is modified to exclude minor components (Modified Max-Min), and one based on a mole-fraction weighted sum of Abraham descriptor differences between all possible mixture

component pairs (Weighted Sum). Results for binary, ternary and quaternary model mixtures as well as for kerosene and Virginia cedar oil are presented and compared. The respective merits of the three approaches are discussed in the context of UVCB mixtures.

Track 2. Ecotoxicology Becomes Stress Ecology: From Populations to Ecosystems and Landscapes

2.01.A Ecological Impacts of Chemical Mixtures and Multiple Stressors: From Evaluation to Prediction

2.01.A.T-01 Chemical Mixtures and Multiple Stressors: Same but Different?

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Ecosystems are strongly influenced by multiple anthropogenic stressors, including a wide range of chemicals and their mixtures. Studies on the effects of multiple stressors have largely focussed on nonchemical stressors, whereas studies on chemical mixtures have largely ignored other stressors. However, both research areas face similar challenges and require similar tools and methods to predict the joint effects of chemicals or nonchemical stressors, and frameworks to integrate multiple chemical and nonchemical stressors are missing. We provide an overview of the research paradigms, tools, and methods commonly used in multiple stressor and chemical mixture research and discuss potential domains of cross-fertilization and joint challenges. First, we compare the general paradigms of ecotoxicology and (applied) ecology to explain the historical divide. Subsequently, we compare methods and approaches for the identification of interactions, stressor characterization, and designing experiments. We suggest that both multiple stressor and chemical mixture research are too focused on interactions and would benefit from integration regarding null model selection. Stressor characterization is typically more costly for chemical mixtures. While for chemical mixtures comprehensive classification systems at suborganismal level have been developed, recent classification systems for multiple stressors account for environmental context. Both research areas suffer from rather simplified experimental designs that focus on only a limited number of stressors, chemicals, and treatments. We discuss concepts that can guide more realistic designs capturing spatiotemporal stressor dynamics. We suggest that process-based and data-driven models are particularly promising to tackle the challenge of prediction of effects of chemical mixtures and nonchemical stressors on (meta-)communities and (meta-)food webs. We propose a framework to integrate the assessment of effects for multiple stressors and chemical mixtures.

2.01.A.T-02 Does Cytochrome P450 inhibition lead to Synergy? A Mechanistic Study of Azole-Pesticide Mixtures in *Enchytraeus crypticus* (Annelida)

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Chemical mixtures can exhibit additive, synergistic, or antagonistic effects. Synergistic interactions are particularly concerning as they amplify toxicity beyond predicted levels. Among known contributors to synergy, azole fungicides play a significant role due to their inhibition of cytochrome P450 monooxygenases (CYPs), essential enzymes in the phase I metabolism of lipophilic pesticides. However, a direct quantitative link between azole CYP inhibition and synergy has not been fully established. This study explores whether in vivo CYP inhibition can predict synergy in binary mixtures of azole fungicides and pesticides (azoxystrobin or cypermethrin). Using *Enchytraeus crypticus* as a model soil organism, CYP inhibition was quantified with an ECOD assay during azole exposure. Dose-response curves for three imidazoles (prochloraz, imazalil, miconazole) and three triazoles (propiconazole, prothioconazole-desithio, epoxiconazole) were constructed. Concurrently, survival dose-response curves were developed for azoles, pesticides, and their mixtures in a 96h survival study. Mixtures were tested using a full factorial design, combining three lethality levels of pesticides (LC10, LC25, LC50) with four inhibition levels of azoles (IC01, IC10, IC20, IC50). Mixture effects were modelled using concentration addition (CA) and observed deviations from model predictions were quantified in model deviation ratios (MDRs). MDRs, indicating synergy (?2) or antagonism (?0.5), were correlated with CYP inhibition to establish predictive

relationships. Preliminary results confirm robust, reproducible CYP inhibition by azoles in *E. crypticus*. Initial survival tests provide accurate LCxx values for pesticides, enabling further synergy assessments. Data will be presented and discussed, linking CYP inhibition and synergistic toxicity. EU-funded Horizon 2020 MSCA-ITN program CHRONIC, Grant Agreement No. 956009

2.01.A.T-03 Synergistic Interaction Between a Toxicant and Food Stress is Further Exacerbated by Temperature

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Global biodiversity is declining at an unprecedented rate in response to multiple environmental stressors. Effective biodiversity management requires a deeper understanding of the relevant mechanisms behind such ecological impacts. A key challenge is understanding synergistic interactions between multiple stressors and predicting their combined effects. Here we used *Daphnia magna* to investigate the interaction between a pyrethroid insecticide esfenvalerate and two non-chemical environmental stressors: elevated temperature and food limitation. We hypothesized that the stressors with different modes of action can act synergistically. Our findings showed additive effects of food limitation and elevated temperature (25°C, null model effect addition (EA)) with model deviation ratio (MDR) ranging from 0.7 to 0.9. In contrast, we observed strong synergistic interactions between esfenvalerate and food limitation at 20°C, considerably further amplified at 25°C. Additionally, for all stress combinations, the synergism intensified over time indicating the latent effects of the pesticide. Consequently, multiple stress substantially reduced the lethal concentration of esfenvalerate by a factor of 19 for the LC50 (0.45 to 0.024 µg/L) and 130 for the LC10 (0.096 to 0.00074 µg/L). Although predicting multiple stressor impacts is challenging, the stress addition model (SAM) proved effective in predicting combined effects. Investigating these interactions with field-relevant pesticide mixtures provides a more realistic approach; therefore, we will also present results on such combined effects.

2.01.A.T-04 Acclimation and Recovery to Pharmaceutical Pollution in a Common Host Fish Under Parasitic Infection

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Freshwater ecosystems face increasing pressure from multiple stressors, including pharmaceutical contamination and biological interactions, yet their combined impacts remain poorly understood. This study investigates the interplay between chemical exposure and parasitism, focusing on the European chub (*Squalius cephalus*), a widespread freshwater fish. Specifically, we examined the effects of paracetamol exposure and acanthocephalan parasite infection on behavior and coloration across acute (16 µg·g⁻¹ for two days), chronic (1.6 µg·g⁻¹ for three weeks), and recovery phases (three weeks). Acute exposure significantly reduced activity and disrupted coloration, while chronic exposure resulted in subtler behavioral changes. Parasitism did not mitigate acute impacts but slightly increased activity during chronic exposure, highlighting complex stressor interactions. Importantly, all measured effects subsided during recovery, indicating the reversible nature of these stressor-induced changes. Our findings emphasize the ecological significance of temporal dynamics and biotic interactions in modulating pollutant effects. By integrating biological stressors, chemical mixtures, and temporal exposure patterns, this research underscores the importance of considering multifactorial influences for accurate risk assessment and ecosystem management. This study was founded by the ANR HELP (ANR-22-CE34-0014), and conducted at the CEREEP Ecotron Ile de France.

2.01.A.T-05 Toxic Heatwave: Chemical Pre-Exposure Alters Springtail Survival in Rising Extreme Temperatures

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Climate change and chemical pollution are accelerating biodiversity loss, emphasizing the need to understand interactions between multiple stressors such as toxic chemicals and extreme temperatures. We investigated this using the Framework of Thermal Death Time analysis (TDT) with 3-week pre-exposed springtails. This was achieved by exposing *Folsomia Candida* (Collembola, Isotomidae), to one control, and two different sublethal treatments of copper and the fungicide fluazianam at a previously found field dose (2 and 10 mg/kg dw soil) at an acclimation temperature of 20 and 24 degrees over 3 weeks. The exposure soils are a LUFA 2.2 standard test soil for the fluazianam exposure and a soil sampled from a copper polluted site in Hygum, Denmark (Former wood treatment facility, with copper sulfate). Subsequently, the animals were exposed to static thermal tolerance assays for increasing exposure times, to generate logistic dose-response curves (survival versus exposure time), for each exposure temperature.

This was used to define how much time is needed to reach 50% mortality (Lt50) at each respective temperature (i.e. a dose of thermal stress). Results showed an effect of acclimation temperature, where 24 °C acclimated animals survived up to twice as long as 20 °C acclimated organisms. Congruently, we found that the two pre-exposure soils strongly affected the springtails' thermal sensitivity where the Lt50 at 33 °C for the 20 °C acclimated animals was 299 minutes (5h) for Hygum soil and 697 minutes (11h) for LUFA soil. This suggests that different soil types drastically change heat sensitivity. Finally, we found the decrease in heat tolerance was larger for fluazinam-exposed animals. This was not the case for 24 °C acclimated organisms where the Lt50 times were not reduced significantly. We also measured the internal concentrations of fluazinam and found increased levels of the parent compound and a hydroxy-metabolite at higher acclimation temperature and the higher exposure concentration. These findings highlight that toxic chemicals significantly alter susceptibility to extreme heat events, with effects modulated by acclimation temperature and soil type. This complexity underscores the challenges for ecological risk assessment and temperature-dependent toxicokinetic-toxicodynamic (TK-TD) modeling in a warming climate.

2.01.B Ecological Impacts of Chemical Mixtures and Multiple Stressors: From Evaluation to Prediction

2.01.B.T-01 Organic Matter Decomposition Responds Differently to Temperature Elevation at Medium Pesticide Concentrations

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The decomposition of allochthonous organic matter is the base of the so-called brown food web, which dominates in headwater streams. Stressors such as pesticides and temperature increase are known to affect organic matter decomposition by impacting associated organisms such as aquatic fungi or macroinvertebrate shredders. To be as close to natural systems as possible, we conducted an outdoor stream mesocosm study with repeated pesticide exposure and two temperature levels to investigate effects on microbial and total (microbially and macroinvertebrate-mediated) decomposition at three time points. We hypothesised that microbial and total decomposition will be reduced with increasing pesticide exposure, while the temperature increase at high exposure levels intensified the reduction but mitigated the effects at low exposure levels. We repeatedly spiked four levels of a realistic mixture containing 13 pesticides were repeatedly spiked to heated (temperature increase ~3.5 °C) and non-heated mesocosms. Per mesocosm, we deployed three fine and coarse mesh bags each filled with black alder leaves (*Alnus glutinosa*, (L.) Gaertn.) for two weeks allowing for microbial and total (microbial and macroinvertebrate) decomposition, respectively, at three time points spanning over two study years. We investigated effects on organic matter decomposition, fungal biomass, exo-enzyme activity as well as fungal communities. In contrast to our expectations, the temperature-normalised microbial decomposition did not vary between any of the different treatments. The absence of a pesticide effect at this concentration level might be caused by permanently altered fungal communities. That the initial fungal communities (July 23) differed between control and high pesticide exposure, while they overlapped later (August 23) points in this direction. The total decomposition only minorly responded to high pesticide exposure, which contrasts previous findings. At medium concentrations, the total decomposition at ambient temperatures differed from the controls for the two August time points, with a strong increase in the second study year. This is possibly related to higher macroinvertebrate feeding rates related to compensation for the single stress of pesticides, while an additional temperature increase disguised this effect. During the conference, we will present and discuss data on the fungal community over all time points, the fungal biomass as well as the fungal exo-enzyme activity.

2.01.B.T-02 Understanding Spatial Drivers of Lake Biodiversity using eDNA

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Biodiversity is negatively impacted by anthropogenic environmental changes such as land use change, chemical pollution and climate change. Freshwater biodiversity in particular provides key ecosystem services yet suffers from river inflow bringing agricultural runoff and urban discharge. Identifying the causes of biodiversity decline across is challenging due to the intricate interplay between biodiversity and environmental stresses. In this study, we used environmental DNA extracted from 52 lakes in England and applied multi-objective optimization explainable multi-view learning, a machine learning approach to discover complex relationships. We identified landscape-level drivers of community biodiversity in lake

ecosystems. Plant protection products, such insecticides and fungicides were identified as the most important factors driving biodiversity dynamics, followed by physico-chemical parameters. Our holistic, data-driven approach provides insights into large-scale biodiversity changes and could inform conservation efforts and regulatory interventions to protect biodiversity from harmful pollutants.

2.01.B.T-03 Interactive Effect of Urban Stressors: Fluoxetine, Light Regime and Noise

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There is a worldwide trend towards urbanization. More and more people are moving to cities, encroaching on surrounding natural areas. Urbanization brings multiple stressors to aquatic ecosystems including chemicals, noise pollution and artificial light at night (ALAN). To date, there is no information on interactions between these three forms of stress. The aim of our study was to investigate the effects of a common chemical contaminant (fluoxetine), ALAN and sudden noise (a motorboat pass by) on the key behaviors in a model fish species, *Danio rerio*. We hypothesized that when a fish's endogenous systems are compromised by external stressors and anthropogenic noise increases the signal to noise ratio for useful auditory information, that their ability to respond to predators will be reduced. In this study zebrafish were exposed to fluoxetine (0 ng/L, 3 ng/L, and 300 ng/L with 4 replicates per treatment; sample size: 11 male: 11 female per replicate) and two light regime (2 replicates: 12 h light: 12 h dark, and other 2 replicates: 12 h light: 12 h 50% of light intensity) for 27 days. Then fish boldness, anxiety, feeding behavior and habitat preference in the presence of a visual predation cue (bird) were measured (recording videos using USB camera) with or without motorboat engine noise using underwater speaker. All recorded behavior videos were analyzed using SwarmSight software. The results showed that fluoxetine alone decreased zebrafish boldness, although effects were dose-sex-dependent. Zebrafish exposed to artificial light at night showed higher activity levels and were bolder than fish that were raised in an environment with a normal photoperiod. Noise exposure often resulted in increased activity. In several experiments, fluoxetine suppressed the effects of artificial light at night, suggesting an antagonistic interaction. Fluoxetine also reduced behavioral responses to sudden noise in several experiments. In the habitat preference test, there was no effect of noise on fish activity in the absence of a predator. However, when the bird was present, noise increased activity by almost 100%. Overall, all three factors affected responses to predation, leading to behaviors that may increase zebrafish vulnerability. This study highlighting the importance of considering multiple forms of stress and their interactions when assessing the effects of urbanization of aquatic systems. The authors thank The Research Council Oman (TRC) (RC/GRG-SCI/BIOL/21/04) grant to AAS and a Sultan Qaboos University internal grant (IG/SCI/BIOL/21/01) to MJB.

2.01.B.T-04 Trojan Horse Mechanism in Soil: Myth or Legend?

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The trojan horse effect, the theory that microplastics (MPs) may adsorb chemicals in the natural environment with the subsequent release of said chemicals upon entering an organism, is often assumed in the terrestrial environment. Although it has been described for the aquatic environment, the assumption that it also happens in the terrestrial environment remains largely unfounded. The literature that is available may provide only slight insight into the complex interactions of MPs with other xenobiotics, as they often consist of binary mixture experiments of metals and MPs, and do not show competition for binding to the MPs. These studies also do not provide insight into the combined effects of co-exposure to other ubiquitous environmental contaminants like poly- and perfluorinated alkyl substances (PFAS). This study uses binary and ternary mixture toxicity experiments with adult *Folsomia candida* to show the interactions of xenobiotics in mixtures with MPs. The lipophilic pesticide chlorpyrifos (Log Kow = 4.96) and the hydrophilic pesticide imidacloprid (Log Kow = 0.57), along with the amphiphilic PFAS perfluorobutane sulfonamide (FBSA) were tested in combination with linear low density polyethylene MPs (10-50µm). Besides the binary mixture toxicity assessments of all combinations of compounds, tests were also performed to examine the ternary mixture toxicity of MPs, FBSA and either of the pesticides, as well as MPs and both pesticides, allowing for the examination of the complex interactions of the different xenobiotics with the MPs, as well as providing insight into the competitive binding of the xenobiotics to the plastic particles. Five replicates of Lufa 2.2 soil containing different combinations of the mentioned compounds were tested at 0.25, 0.5, 1, 2, and 4 toxic units (TUs), based on previously determined EC50 values for reproduction. This allowed us to evaluate the full effect plane, including synergism and antagonism, but also dose-ratio and dose-level dependent effects of the binary and ternary mixtures. Although the data has all been collected now, the analysis is ongoing and results will be presented during the meeting in Vienna. Preliminary data shows compound-specific interactions with the MPs. This study will therefore provide valuable insight not only in the ecotoxicological effects of these mixtures, but also

on complex mixture toxicity itself. This research was funded by the PAPILLONS project, European Union's Horizon 2020 Research and Innovation Programme: Grant Agreement Number 101000210; <https://www.papillons-h2020.eu/>, and by the Open Technology Program of the Netherlands Organization for Scientific Research (NWO), domain Applied and Engineering Sciences (TTW) under project number 18725.

2.01.P-Mo119 Assessing the Toxicity of a Mixture of Benzotriazole Ultraviolet Stabilizers to Zebrafish (*Danio rerio*): Insights into Embryotoxic and Molecular Effects

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Benzotriazole ultraviolet (BUV) stabilizers are a group of industrial chemicals used in industrial (e. g. textiles, plastic, building materials) and personal care products (e.g. sunscreens, body lotions, hair dyes) to protect materials or skin from damage against harmful UV-radiation. They are considered a class of emerging environmental contaminants, although they have been around for nearly six decades and they can absorb UV rays from sunlight (within the 280-400 nm spectrum). BUVs (e.g., UV-P, UV-326, UV-327, UV-328, and UV-329) have been detected in different marine compartments worldwide, such as marine waters and sediments. A variety of marine animals, from small crustaceans to large mammals, have accumulated BUVs. BUVs have been detected in aquatic environments at concentrations ranging from 0.05 to 99,200 ng/L; however, there are few studies on the effects of BUVS mixtures on aquatic organisms. Studies show that BUVS can affect behavioral responses and acetylcholinesterase activity, indicators of neurotoxicity. Additional studies support the hypothesis that BUVS induce oxidative stress and neuroinflammation in fish. The objective of this study was to evaluate the embryotoxicity of a mixture of BUVS and to use whole transcriptome sequencing to link toxicities with effects. We selected 5 UVBs including UV-326, UV-327, UV-328, UV-329, and UV-P for toxicological analysis, as these are among the most frequently detected in aquatic environments. Using microinjection, freshly fertilized embryos were exposed zebrafish embryos to 3 different doses of the BUVS mixtures before gastrulation, and effects on mortality, hatch success, heart rate, and malformations were evaluated until 14 days post-fertilization. We observed an increase in mortality dependent on the dose of BUVS, for the control group mortality was 2%, and 15, 23, and 33 % for the groups treated with BUVS. Likewise, BUVS caused a decrease in heart rate at 48 hours post-fertilization, the control group presented an average of 161 beats per minute (bpm), while the treated groups averaged 159, 157, and 156 bpm. Also, the larval size, eye area, yolk area, and melanophores in the head area in the treated groups decreased compared to the control group. Whole transcriptome (mRNA, non-coding RNAs) analysis is currently being performed to determine the molecular basis of the observed effects. This study provides new insight into the toxicity and hazards of aquatic organisms.

2.01.P-Mo130 CheOSmix - A Data-Reuse Approach to Determining Chemical Mixture Risk Driver Heterogeneity in European Freshwater

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Chemical pollution of aquatic environments involves complex combinations of substances. Mixture exposure can provoke combined effects even if the concentrations of individual substances remain below their effect thresholds. Therefore, efforts are ongoing to better consider mixture effects in chemical safety regulation. A debate accompanies these efforts on whether only a few substances drive the aquatic mixture risk and, thus, whether more strictly regulating these individual substances would be sufficient or whether a large heterogeneous set of driver substances needs to be considered. Here, we employed a data-reuse strategy to investigate the heterogeneity of drivers of chemical mixture risk to aquatic species. We initially demonstrate that drivers are heterogeneous using data from a single measurement campaign that enables comparing drivers between sites due to a set of consistently measured substances. Subsequently, we extended our analysis to an extensive collection of chemical monitoring data from the NORMAN network. These data originate from different measurement campaigns that focused on diverging sets of substances at various time points. We, therefore, aggregated the data quarterly and per quarter cluster sites according to the set of measured substances. Our study concludes that an extensive list of substances drives chemical mixture risk across Europe and that these substances were highly heterogeneous between

locations. Notably, the drivers of mixture risk were species-specific and exhibited variability over time. Also, we established that lacking monitoring data hindered a more precise analysis, particularly regarding temporal variability. We have stored all collected and curated data in a graph database. We present a chatbot that builds on a graph database - grounded large-language AI model to translate between the researcher and our database and, therefore, allows querying this knowledge for non-computer science experts.

2.01.P-Mo155 Co-occurrence of Microplastics and Contaminants of Emerging Concern in River Water, Sediment and Fish: The Case Study of Júcar River basin (Spain)

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There is rising concern about the environmental pollution by microplastics (MPs), especially about their ability to accumulate various types of contaminants on their surface. These contaminants include pesticides, pharmaceutical and personal care products (PPCPs), per-/ poly-fluoroalkyl substances (PFASs) and polycyclic aromatic hydrocarbons (PAHs). The co-occurrence and combined toxicity of MPs and organic pollutants needs to be studied. Thus, the presence of MPs, pesticides, PFASs, PPCPs and PAHs in water, sediment and fish samples from the Júcar River basin (Spain) was determined. The Júcar River flows through three different provinces, being one of the most important rivers in E Spain. The extraction was optimized for each matrix and contaminant. Density separation and digestion were combined for MPs extraction from water and sediment, while a microwave-assisted digestion was performed for MPs present in the fish gastrointestinal track. For the organic pollutants, solid-phase extraction and microwave-assisted extraction were adapted for each matrix. For MPs, ¹HFTIR and Pyrolysis coupled with Gas Chromatography-Mass Spectrometry (Py-GC-MS) were utilized, while the PAHs were analysed using GC-MS and the rest of the contaminants were analyzed by High Pressure Liquid Chromatography-High Resolution Mass Spectrometry (HPLC-HRMS/MS), in both target and non-target modes. ¹HFTIR indicated the presence of MPs in all water samples (0.1- 6.9 MPs L⁻¹), whereas 73% of the sediment samples and 66% of the fish samples were polluted by MPs (sediments: 20- 140 MPs Kg⁻¹, fish: 6.7- 26.7 MPs g⁻¹). The main polymer types were polyethylene (water and sediments) and polyvinyl chloride (fish). Tebuconazole and difenoconazole were the most abundant pesticides in water. Perfluorobutane sulfonate and the painkillers ibuprofen and tramadol, were found in all water samples. The initial water analyses points to correlations between MPs and pesticides (i.e. propazine, imazalil, thiabendazole) and the flame retardant tris(chloropropyl) phosphate, particularly at two sampling points (highest MP amounts). Completing all analyses of sediment and fish samples will provide a better understanding of MPs and emerging contaminants co-occurrence. The risk assessment that is currently being performed, allows the estimation of the environmental toxicity that multiple anthropogenic pressures cause to wildlife and human health in a basin representative of the Mediterranean. This work has been supported by the Generalitat Valenciana (GV) through the Prometeo Programme CIPROM/2021/032. V. Sourso also acknowledges her pre-doctoral contract ACIF/2021/408 and the grant CIBEP/2023/154, both funded by the GV. The latter allowed her to work at the University of Pisa, Italy for 3 months.

2.01.C Ecological Impacts of Chemical Mixtures and Multiple Stressors: From Evaluation to Prediction

2.01.C.T-01 Bioenergetic Responses Shape the Impacts of Pharmaceuticals and Warming on Freshwater Arthropods and Ecosystem Functioning

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Freshwater ecosystems face growing threats from pharmaceutical contaminants (PhACs) and climate change-induced warming. However, the combined effects of these stressors on freshwater taxa remain poorly understood, particularly the mechanisms connecting (sub)individual responses to broader ecological impacts. We explored the effects of environmentally realistic concentrations of PhACs and warming on two aquatic arthropods, *Asellus aquaticus* (Isopoda) and *Cloeon dipterum* (Ephemeroptera), in outdoor pond mesocosms during winter and summer. By assessing physiological traits (bioenergetic responses and oxidative damage) alongside population densities and ecosystem functions, we identified seasonal patterns in sensitivity to both stressors. In winter, PhACs reduced energy availability and increased oxidative damage, while in summer, PhACs elevated energy availability and decreased

oxidative damage. Ecosystem functions, such as leaf litter decomposition and insect emergence, reflected population- and bioenergetic-level responses. Reduced leaf litter decomposition was linked to declining *Asellus* abundance under combined stressors, whereas decreased *Cloeon* emergence in summer was attributed to PhAC-induced fitness reductions. Warming alone consistently reduced arthropod abundances and emergence, except for increased *Asellus* abundance in winter. Crucially, the bioenergetic-mediated effects of these stressors outweighed their direct impacts. This study underscores the vulnerability of aquatic arthropods to PhACs and warming, highlighting consequences for energy metabolism, population dynamics, and ecosystem functioning. These findings emphasize the need for integrated strategies to mitigate the ecological impacts of emerging contaminants and climate change.

2.01.C.T-02 Integrating Statistical Models and Multiple-Stressor Null Models: A Framework for Analysing Stressor Interactions

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Understanding how environmental and toxicant stressors interact is crucial in multiple-stressor and ecotoxicological research. Stressor interactions are identified as deviations from theoretical null models that describe how stressors combine. Selecting an appropriate null model is a critical step and should be grounded in theory. In the co-tolerance framework, three distinct null models are used to characterize combined stressor effects. They assume that stressors act independently, but they differ in their assumptions on how stressor sensitivities correlate within a community or population: the multiplicative model (no correlation), the simple addition model (negative correlation), and the dominance model (positive correlation). To evaluate the magnitude to which joint stressor effects deviate from null model predictions, the model deviation ratio is commonly used. Fixed thresholds are used to identify interactions; however, this approach does not account for empirical uncertainty. Alternatively, regression models can evaluate statistical significance of interaction terms, however, they introduce constraints by imposing a specific null hypothesis on stressor combinations that cannot flexibly be changed by the analyst. To address these challenges, we present a theoretical framework that (1) clearly communicates which regression model specification aligns with which null model and (2) uses post-estimation hypothesis testing to evaluate a specific null model, if regression results cannot directly be used. Interaction estimates can be calculated from adjusted model predictions, and associated standard errors are derived using either the delta method, posterior simulations or bootstrapping. This approach enables analysts to flexibly test different null models independent from regression model structure, ensuring robustness in describing empirical patterns. We illustrate our approach with a case study and validate the calculated confidence intervals through data simulations. This work is funded by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation: CRC 1439/1 (RESIST) [grant number 426547801]).

2.01.C.T-03 Probabilistic Risk Assessment of Chemical Mixtures: Cumulative Effects and Joint Risk of Pesticides in Freshwater Biological Communities

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We have developed a hierarchical probabilistic model (object-oriented Bayesian network - BN) for exploring ecological risk of pesticides mixtures to non-target biological endpoints in freshwater ecosystems. Our study is based on a monitoring dataset for 10 stream sites in Germany, with three pesticides per site, in total 13 different pesticides. Traditional environmental risk assessment (ERA) approaches are largely based on single-substance and single-species testing. Two major challenges for more realistic ERA are therefore assessing chemical mixtures and community-level responses. Modelling methods developed for handling these challenges typically aim to reduce the complexity of the chemical stressors (e.g., a Mixture Assessment Factor) and of the community response. Our BN model, in contrast, incorporates an empirical dose-response relationship for each pesticide-to-endpoint pathway. For each pesticide, we used the model PERPEST to predict the probability of three alternative effect classes (none, slight, clear) for up to 10 biological endpoints, using a database of mesocosm studies and case-based

reasoning. We then used the BN to aggregate the effect probabilities from single pesticides, via pesticide groups with similar mode of action, to mixtures by joint probability (Boolean or) expressions. Furthermore, the BN aggregates the probability of effect classes from the level of individual endpoints (e.g., algae, rotifers, insects), to endpoint groups (e.g., plants, zooplankton, macroinvertebrates), and subsequently to the community level. This BN enables exploring of pesticide risks from different angles by joint probability calculation in combination with alternative assessment rules. For example, at a given site, we can address questions such as: (1) What is the probability that at least one pesticide will have a clear effect on at least one biological endpoint? (2) Which endpoint groups are most likely to be affected by the observed pesticide mixture? (3) Which group of pesticides contribute most to community-level risk? This work originates from the NORMAN Network workshop "Improving the use of (semi-)field data for the risk assessment of chemicals" (WG2 Bioassays), organized in November 2022 by the Expert Group on Ecosystem level effects of chemicals of emerging concern on aquatic ecosystems. The pesticide monitoring data were introduced to the workshop by Matthias Liess.

2.01.C.T-04 Derivation of Field-Based Macroinvertebrate Sensitivities and Stressor-Specific Indicators

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Aquatic ecosystems are exposed to multiple stressors. A large number of metrics and indicators exist to describe their effects. While common stressor-integrating metrics (e.g. ecological quality ratio) allow a general description of ecological status, stressor-specific indicators (e.g. saprobic index, SPEARpesticides) necessary for effect attribution are rare. Therefore, we have developed a method to derive specific indicators for a variety of environmental stressors based on field-based estimates of macroinvertebrate taxa sensitivities. Based on a comprehensive stream monitoring data set from 101 stream sections, including measurements of various environmental and anthropogenic stressors and macroinvertebrate abundances, we fitted dose-response curves and derived median effective doses (ED50s) for each taxon and stressor. Subsequently, we calculated the empirical SPEAR (empirical SPecies At Risk, e-SPEAR) bioindicator, which represents the abundance-weighted mean ED50 of the macroinvertebrate community to one stressor. Indicator performance and specificity was assessed using single and multiple linear regressions. We were able to derive ED50s for 139 macroinvertebrate taxa and 15 stressors. Taxa sensitivities were highly correlated for individual stressor combinations (Pearson correlation, up to $r > 0.50$ or $r < -0.50$), indicating the existence of generally more or less sensitive species. Of the 15 e-SPEAR indicators developed, 11 showed significant ($p < 0.05$) linear regressions with the respective stressor values, and 8 indicators performed well ($R^2 > 0.20$). The indicators performed best for pesticide toxicity, oxygen, hydromorphology and nitrate ($R^2 > 0.30$), followed by ammonium, flow velocity, pH and grabby substrate. This ranking may indicate stressor importance, as more dominant stressors would produce more pronounced community dose-response relationships. The multiple regressions revealed that the stressors corresponding to the e-SPEARs had the highest individual contribution to the total explained variance (39% to 94% of R^2). Accordingly, although taxa sensitivities and stressor gradients were highly correlated, we were able to generate indicators with a high stressor specificity. The indicators allow a specific attribution of effects to individual stressors, which is essential for targeted monitoring and management.

2.01.C.T-05 Sequential Toxicity of Pesticides to *Enchytraeus Crypticus*: Using Toxicokinetic–Toxicodynamic Modelling

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Considering the toxicity of pesticides in scenarios which relate to those seen in the environment is important in determining the risk posed to organisms. Although the presence of multiple chemicals in mixtures is acknowledged in toxicity studies, there may be a sequential timeline of exposure due to application timings. The time between exposure pulses may allow for recovery, be it toxicokinetic via excretion and metabolism or toxicodynamic via damage repair. However, if a subsequent exposure pulse occurs before recovery then carry-over toxicity from the first chemical may occur. Here we assess the effects of sequential exposure of *Enchytraeus crypticus* to combinations of pesticides in acute aquatic exposures. Cypermethrin along with one of three other pesticides, chlorothalonil, azoxystrobin or prochloraz, were applied in sequence separated by a recovery period. In all cases there was a sequence effect seen. When cypermethrin was applied during the first pulse, the slow recovery caused carry-over toxicity and higher mortality by the end of the study, this contrasted with exposure to the same

combination in the reverse order. Chemical analysis supported that the short recovery period used did not allow for complete toxicokinetic recovery to cypermethrin, as internal concentrations were still elevated during the second pulse period, hence simultaneous exposure was present for some period during these studies. Using toxicokinetic-toxicodynamic modelling to predict the effects of the sequential studies indicated the importance of understanding the carry-over effects following pulse exposures, as the cypermethrin predictions did not accurately align with the delay period seen before toxicity effects are observed. At some concentrations synergism leading to toxicity above that predicted may also play a role in the difference between observed and predicted toxicity in the model. The occurrence of both carry-over toxicity and sequence effect in these studies highlight the importance of considering the potential for sequential exposure when determining the risk of pesticide mixtures.

2.01.P Ecological Impacts of Chemical Mixtures and Multiple Stressors: From Evaluation to Prediction

2.01.P-Mo109 Long-term Trends for Biodiversity in a Multi-Stressor Environment

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The triple planetary crisis chemical pollution, biodiversity loss, and climate change poses significant challenges for environmental management and monitoring. Recent advances in environmental chemistry and biology have generated unprecedented data, yet their transformative potential remains largely unused due to a lack of standardization, expertise, and resources, as well as sociological barriers. National Environmental Specimen Banks (ESBs), established in the late 20th century, have pioneered systematic sampling and archiving of biological and non-biological matrices to support chemical monitoring, review the effectiveness of chemicals management and enable retrospective and future research. The German ESB, one of the most advanced of its kind, has archived over 500,000 subsamples from terrestrial, freshwater, and coastal ecosystems over four decades. 15 species, including fish, molluscs, terrestrial vertebrates, seabirds, tree leaves, suspended matter and soils, were continuously sampled according to strict protocols. Uniquely, the cold chain begins in the field after sampling with the samples being stored over liquid nitrogen. Samples are cryo-archived at temperatures below -150°C , preserving their integrity for comprehensive analyses. These samples have been extensively characterized for pollutants (e.g. organic pollutants as well as metals and chlorinated, brominated and fluorinated pollutants) and tested using innovative approaches, including high-resolution mass spectrometry (HRMS) non-target screening (NTS), transcriptomics, and eDNA metabarcoding as well as a wide range of classical ecotoxicity tests. These methods have enabled retrospective tracking of biodiversity changes, invasive species occurrences, and population genomics trends, offering insights into the interplay between chemical pollution and biodiversity shifts. The German ESB demonstrates the potential for integrated monitoring that links chemical and biodiversity data to understand environmental changes and predict future trends. The bank's samples and data are available to the scientific community, fostering collaborative research to inform chemical management, environmental monitoring, and nature conservation policies. These efforts contribute to advancing integrated environmental assessment and addressing the challenges of the planetary crisis.

2.01.P-Mo110 Defining a Safe Operating Space for European River Biodiversity in the Context of Multiple Stressors – DESTRESS Project

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The triple planetary crisis climate change, chemical pollution, and biodiversity loss represents an interconnected challenge with profound implications for river ecosystems. These factors affect not only freshwater ecosystems biodiversity but also the management of water resources, for human consumption in terms of quantity and quality. In this context, the DESTRESS project aims to understand how the interactions between multiple stressors flow regimes, chemical pollution, and land use affect river biodiversity. By identifying the thresholds and boundaries for ecological resilience, DESTRESS seeks to support sustainable management strategies tailored to current challenges. In DESTRESS, we use long-term biodiversity time-series on river macroinvertebrates collected across Europe and environmental data to relate biodiversity trends to extreme hydrological events and chemical contamination. This transdisciplinary approach integrates macroinvertebrate inventories, flow metrics, and toxicity models

across geographical regions within Europe. Data science and machine learning techniques will be applied to analyze large datasets, and identify patterns and resilience thresholds of biodiversity to anthropogenic stressors. Additionally, direct collaboration with water managers and authorities at various levels will ensure that the analyses focus on actionable solutions for water management and biodiversity conservation. The expected results will provide a deeper understanding of the critical thresholds beyond which stressors and hydrological pressures begin to cause biodiversity losses. These results will provide recommendations for water managers on ecologically acceptable thresholds to protect biodiversity while ensuring sustainable water resource management. Ultimately, the DESTRESS project aims to provide Safe Operating Spaces as operational tools for more resilient and adaptive environmental policies.

2.01.P-Mo111 Effect of Cumulative Municipal Wastewater Exposure on Benthic Macroinvertebrate Assemblages: An Experimental Stream Approach

Aphra Mary Sutherland, Frederick Wrona and David Barrett, University of Calgary, Canada

Municipal wastewater effluent (MWE) is a common source of nutrient enrichment in urban waterways but, increasingly, also provides a route for many pharmaceuticals and other chemicals not targeted by wastewater treatment to be released into aquatic systems. Benthic macroinvertebrates community endpoints such as species diversity metrics and changes in composition have been utilized to assess responses to nutrient enrichment, but these endpoints are not well characterized in systems where MWE contains increasingly diverse chemical mixtures and in systems with contributions from consecutive wastewater treatment plants (WWTPs). Community endpoints may be better evaluated for complex MWE mixtures in the environment in artificial stream systems where MWE exposure can be controlled, while maintaining elements of the in-situ receiving environment. An assessment of a range of benthic macroinvertebrate community endpoints was performed at the Advancing Canadian Water Assets facility in Calgary, Canada using a control stream (Bow River water with background MWE) and a treatment stream with an additional 5% of total stream volume input of treated MWE. Benthic macroinvertebrate communities characterized by conventional area-limited Surber samples showed no differences between control and treatment streams. In contrast, when using more highly controlled artificial substrate samplers, exposed in the streams for six weeks, displayed significantly lower taxonomic diversity and evenness in the treatment streams, while the relative contribution of the dominant taxa was significantly higher. No additional measure of community assemblage characterization, such as raw abundance or distribution of major taxonomic groups, showed differences between the two streams, suggesting that non-nutrient components of MWE, even when combined from cumulative MWE exposures, are not associated with observed impacts at the community level. Additionally, differences in the artificial substrate samplers suggest that colonizing communities may be more sensitive to cumulative MWE exposure than established communities, which may affect the long-term resilience of the stream. This study emphasizes the need for biomonitoring programs to consider using multiple approaches to assess impacts of complex urban pollutants.

2.01.P-Mo112 Basal Autotrophic and Heterotrophic Food Web Responses to Municipal Wastewater Effluent Exposure

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Municipal wastewater effluent (MWE) release into urban rivers act as a main point source of nutrients and increasingly the addition of environmental substances of concern (ESOCs), such as pharmaceuticals and personal care products, which can alter the physiology and ecology of aquatic organisms. While there have been active efforts to reduce the deleterious effects of MWE loading on river systems, our understanding of nutrient-ESOC interactions and potential impacts on basal components of the food web is relatively limited. To address this, experimental streams at the Advancing Canadian Water Assets (ACWA) in Calgary, Alberta, Canada, were used to examine the responses of basal autotrophic and heterotrophic communities to a controlled incremental gradient of MWE exposure over six weeks. Three stream treatments were deployed including a control (the nearby Bow River water only - BR), a 5% (v/v) MWE addition (PC5), and a 15% (v/v) addition (PC15). An integrated suite of controlled bioassays incorporating artificial substrates (rock baskets), nutrient diffusing substrata, and cotton strips were used to assess the effects of increasing MWE concentrations on algal standing crop and heterotrophic production, nutrient limitation, and organic matter decomposition rates. Increased MWE exposure concentration was found to suppress autotrophic standing crop. Nutrient-ESOC interactions and potential grazing effects from macroinvertebrates were identified as a potential cause for the patterns observed. Heterotrophic production (via biofilm biomass) showed a subsidy-stress pattern, where PC5 had increased biomass compared to BR and PC15 treatments. However, the elevated MWE concentration had no impact on organic matter decomposition rates. Relative autotrophic and heterotrophic biomass (measured via autotrophic index) showed increasing MWE concentrations produced a shift towards

more heterotroph dominant biofilm communities with time. The consistent inhibition of autotrophic endpoints highlights the relative MWW impacts and influence of nutrient-ESOC interactions to autotrophs were greater than that of heterotrophs, introducing implications to efficient energy transfers to higher trophic levels. This study exemplifies the significance of utilizing integrated bioassays in experimental controlled streams to identify how nutrient-ESOC interactions influence autotrophic and heterotrophic biofilm communities and function in urban rivers.

2.01.P-Mo113 Effect of Co-exposition of Cadmium and Phthalate on Development of the Insect Agricultural Pest *Spodoptera Littoralis*

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Our environment is increasingly polluted with various molecules, some of which are considered endocrine disruptors. Metals and phthalates, originating from industrial activities, agricultural practices, or consumer products, are prominent examples of such pollutants. We experimentally investigated the impacts of the heavy metal cadmium and the phthalate DEHP on the moth *Spodoptera littoralis*. More specifically, larvae were reared in laboratory conditions, where they were exposed to diets contaminated with either two doses of cadmium at concentrations of 62.5 µg/g or 125 µg/g, two doses of DEHP at 100 ng/g and 10 µg/g, or a combination of both low and high doses of the two compounds, with a control group for comparison. Our findings indicate that cadmium delays the developmental transition from larva to adult. Notably, the combination of cadmium and DEHP exacerbated this delay, highlighting a synergistic effect. In contrast, DEHP alone did not affect larval development. Additionally, we observed that cadmium exposure, both alone and in combination with DEHP, led to a lower mass at all larval stages. However, cadmium-exposed individuals that reached adulthood eventually reached a similar mass to those in other groups. Interestingly, while our results did not show any effect of the treatments on hatching success, there was a higher adult mortality rate in the cadmium-treated groups. This suggests that while moths may prioritize reproductive success, their survival at the adult stage is compromised by cadmium exposure. In conclusion, our study demonstrates the impact of cadmium on the development, mass, and adult survival of moths, and reveals synergistic effects when combined with DEHP. These results confirm cadmium as an endocrine disruptor, even at low doses. These insights underscore the importance of understanding the toxicological effects of low doses of pollutants like cadmium and DEHP, both individually and in combination.

2.01.P-Mo114 Zebrafish Physiological and Sub-Cellular Changes under the Combined Exposure of the Nanomaterial Mg-Al LDH and the Antineoplastic Epirubicin

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Engineered nanomaterials like layered double hydroxides (LDH) and antineoplastic agents (AA) are two examples of emerging contaminants (EC) that can be found in the aquatic environment. LDH is applied across various fields, from catalysis to drug delivery, and the chances of ending up in the aquatic environment are high. AA can affect organisms even at low concentrations, and they have already been detected in aquatic environments. These two EC can end up in water bodies originated from different sources: independent usage (each used in different applications) or under a combined use (e.g. drug delivery system for AA). Still, the knowledge about their possible harmful effects is almost null, and it is essential to determine their single and combined toxicity. This study evaluated the single and joint toxicity of Mg-Al LDH and Epirubicin in *Danio rerio*. *D. rerio* eggs were exposed to a range of concentrations of Mg-Al LDH (13 to 100 mg/L) and Epirubicin (11.46 to 87 mg/L; 41 to 206 µg/L) for single exposure. A full factorial design was applied using the same concentrations for mixture exposure. After 96 hours of single and mixture exposure, according to the OECD 236 FET protocol, mortality, malformations, and hatching delay were documented. Biochemical markers and DNA damage were also assessed. No mortality or malformations were observed at the concentrations tested of Mg-Al LDH. Epirubicin showed an LC50 of 49.3 mg/L (CI=45.5 53.0) and a NOEC < 11.5 mg/L based on malformations. The mixture exposure showed no alterations in the LC50 of Epirubicin with the increasing concentrations of LDH, ranging between 46.9 to 59.9 mg/L. In single exposure, Mg-Al LDH didn't cause genotoxicity, while Epirubicin induced 20-30 % DNA damage at concentrations higher than 92 µg/L and biochemical alterations were only observed after 120 h of exposure to Epirubicin at a concentration of 206 µg/L. After mixture exposure, no genotoxicity was observed, however biochemical markers results suggest activation of the antioxidant defense system as indicated by an increased catalase (CAT) and glutathione peroxidase (GPx) activities. Furthermore, no alterations were observed in other enzymatic biomarkers, suggesting that Mg-Al LDH can mitigate the sub-cellular effects caused by Epirubicin on zebrafish larvae. These findings provide valuable insights into the effects promoted by mixtures of EC and may assist in their future

environmental hazard and risk assessment. The authors thank for the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020+LA/P/0094/2020) to FCT/MEC through national funds. FCT also financed a doctoral grant to Diana Carneiro (2021.06691.BD; DOI: 10.54499/2021.06691.BD) and a researcher grant to R. Martins (2021.00386.CEECIND; DOI: 10.54499/2021.00386.CEECIND/CP1659/CT0011). The present work was supported by the project NANOGREEN (CIRCNA/BRB/0291/2019); DOI: 10.54499/CIRCNA/BRB/0291/2019 and VitroTox (PTDC/CTA?AMB/0126/2020; DOI: 10.54499/PTDC/CTA-AMB/0126/2020), financed by national funds (OE), through the Portuguese Foundation for Science and Technology (FCT). We also acknowledge the financial support of the Compete 2030/FEDER to the NANOBIOESCUDO project (COMPETE2030-FEDER-01194000) and from the European Union's Horizon programme under the grant agreement No 101182588 through the project SAFERCOAT.

2.01.P-Mo115 The Acute Oral Toxicity of Pesticide Mixtures Identified in Agricultural Fields to Honey Bees (*Apis mellifera* L.)

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Honey bees (*Apis mellifera* L.) are essential pollinators for crops and therefore may spend a significant amount of time near agricultural fields, putting them at risk for pesticide exposure. The effects of pesticides on honey bees are well documented, although most research focuses on single compounds. However, honey bees are exposed to multiple pesticides in the environment while foraging, illustrated by the mixtures of pesticides measured in hive matrices. The objective of this study is to assess the toxicity of pesticide mixtures to honey bees and compare this to predicted toxicity to explore possible interactive effects. We conducted an acute oral toxicity test on honey bees for 11 pesticide mixtures, each comprised of 3-5 compounds of various modes of action, which were identified in soils of agricultural fields of 10 European countries and Argentina. Honey bees were exposed to several concentrations of each mixture and mortality was recorded every 24 hours for 96 hours. We aim to predict the toxicity of these mixtures with concentration addition models, utilizing existing acute oral toxicity data for the single components. Predicted and measured toxicities will be compared to uncover additive or synergistic effects. In particular, mixtures containing groups such as azole fungicides, which have been commonly observed in synergistic mixtures, may exhibit greater differences between measured and predicted toxicities. These results will contribute to an improved understanding of the potential interactions of pesticide mixtures detected in agricultural environments, and how such mixtures may affect honey bees that come into contact with them. This research has received funding from the European Union's Horizon 2020 Programme for research & innovation under grant agreement no. 862568 (SPRINT).

2.01.P-Mo116 Genotoxic Effects of Three Anticancer Drug Mixtures to Zebrafish

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Across the world, millions of patients are undergoing chemotherapy, resulting in an increased consumption of anticancer agents (AA). Once administered to patients, these substances often end up in the environment due to the inefficiency of wastewater treatment plants in fully removing them. Therefore, AA typically coexists with other AA and/or contaminants, thus increasing the need to evaluate the potential effects of such mixtures on non-target aquatic species. This study evaluated the single and joint genotoxic effects of three AA, trabectedin (TRAB), doxorubicin (DOX), and oxaliplatin (OXA), commonly used in cancer treatment patients jointly or in sequence, using the zebrafish model, *Danio rerio*. Embryos of *Danio rerio* were exposed to the three AA individually and in combination using sub-lethal concentrations based on previously performed assays. The single-cell gel electrophoresis (comet) assay was performed to evaluate DNA strand breaks in *D. rerio* exposed to both individual and joint exposures after 96 hours. The Independent Action (IA) model was applied as a reference additivity model to detect and evaluate the interactions (synergism or antagonism) between the three AA. The predicted mixture effects were calculated using the control-normalized single-treatment responses and compared to the observed mixture response. Whenever the mixture effects were assumed to be non-additive the interaction type, strength, and direction were analysed. The results show that both TRAB-DOX and DOX-OXA mixture treatments exhibited lower-than-predicted DNA damage (one-directional antagonism up). The TRAB-OXA mixture showed the opposite (one-directional synergism up), with the exception of the combination of the lowest TRAB and highest OXA concentrations that revealed lower DNA damage than expected (antagonism up). The results of this study highlight the importance of assessing the effects of AA

mixtures in the environment rather than solely focusing on their individual effects. Additionally, it provides insights for a better consensus on synergistic or antagonistic operational interactions. The present study was funded by VitroTox (PTDC/CTA?AMB/0126/2020; DOI: <http://doi.org/10.54499/PTDC/CTA-AMB/0126/2020>) supported by the FCT, in its State Budget component (OE). Daniel Bruno received funding (BI/UI88/10456/2022) via the VitroTox project. Thanks are due for the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020+LA/P/0094/2020), to FCT/MEC through national funds.

2.01.P-Mo117 Disentangling the Web: The Impacts of Binary Chemical Mixtures on Consumer-Resource Feeding Interactions

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Current evaluation of the impacts of chemicals and their mixtures on freshwater ecosystems relies heavily on single- and multi-chemical effects on individual species. However, species do not exist in isolation, and a mechanistic understanding of how chemicals and their mixtures impact species interactions is essential if we are to extrapolate from single-species single-chemical studies to assessing the impact of multiple chemicals on multispecies assemblages in ecosystems. Consumer-resource interactions, which represent fundamental building blocks of the food webs that drive biodiversity, may be impacted by chemicals through changes in abundance (e.g., lethal effects) or through sub-lethal effects such as changes in feeding behaviour, escape response or mobility. The chemicals in a mixture may affect consumer-resource interactions by affecting either the resource, the consumer, or both the resource and the consumer to the same or different extents. Here we demonstrate the use of a consumer-resource system to begin to disentangle the complex effects of chemical mixtures on ecological communities. We present the results from a series of crossed-design binary-chemical consumer (grazer)-resource (primary producer) interaction experiments using the freshwater snail, *Potamopyrgus antipodarum* and the freshwater diatom, *Nitzschia palea*. The chemical mixture consisted of a biocide (copper) and insecticide (azoxystrobin) and the experiments addressed three questions: (1) How are the impacts of binary mixtures altered depending on whether they are acting directly on an organism or indirectly through the consumer-resource interaction? (2) What are the consequences of differential sensitivity to binary mixtures for consumer-resource interactions? (3) Do the combined effects of individual chemicals within binary mixtures exert their impacts additively or otherwise in the case of consumer-resource interactions? This studentship is funded by the United Kingdom Natural Environment Research Council (NERC) and Unilever.

2.01.P-Mo118 First Analysis of the Application of Health Impact Assessment in Italy: The Role of Effect-Based Methods in Mixtures Assessment

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The Health Impact Assessment (HIA) as defined by WHO is a practical approach used to systematically judge the potential health effects of a policy, strategy, plan, programme or project on a population. In Europe, the Environmental Impact Assessment (EIA) Directive 2014/52 foresaw that an estimate of the potential public health impacts of the implementing project must be carried out in every EIA procedure. In Italy the HIA has been applied to new plants belonging to the categories of large combustion plants, crude oil refineries, re-gasification and liquefaction plants or for plants that can have strong impacts in relation to their production activity. These plants can release in air, soil and waterbodies several chemical contaminants in low quantities. However their interaction can generate mixtures whose effects can be synergistic with possible impacts for ecosystems and human health. The use of Effect-based Methods (EBM) represents a key aspect for the evaluation of the effects of mixtures in the ecosystems. In this work a first 5 years analysis of the application of HIA in Italy has been carried out. Specifically, 42 industrial projects were analysed, distributed as follows: 50 % in the Northern, 9% in the Central and 41% in the Southern Italy. The projects examined included 81% Thermoelectric Power Plants, 17% Regasification plants and 2% Biorefineries. In this context EBM have been applied mainly to surface inland waters, transitional and marine waters and soils. The bioassays applied covered different trophic levels and also endpoints such as genotoxicity and embryotoxicity. They are mainly in vivo bioassays acute and chronic (e.g., test with earthworms, algae, crustaceans, fish embryos); eco-genotoxicological assays such as the Ames test, Comet assay and the Micronucleus test have been carried out. When effects such as genotoxicity and embryotoxicity have been detected, specific actions have been applied: an extensive chemical monitoring plan of the area and a trend analysis to investigate the causes and apply risk reduction measures. The results of this 5 years analysis showed that the inclusion of effect-based methods in the HIA procedure will contribute to apply the appropriate preventive measures needed to reduce the impacts on the population of the industrial chemical emissions and releases.

2.01.P-Mo119 Assessing the Toxicity of a Mixture of Benzotriazole Ultraviolet Stabilizers to Zebrafish (*Danio rerio*): Insights into Embryotoxic and Molecular Effects

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Benzotriazole ultraviolet (BUV) stabilizers are a group of industrial chemicals used in industrial (e. g. textiles, plastic, building materials) and personal care products (e.g. sunscreens, body lotions, hair dyes) to protect materials or skin from damage against harmful UV-radiation. They are considered a class of emerging environmental contaminants, although they have been around for nearly six decades and they can absorb UV rays from sunlight (within the 280-400 nm spectrum). BUVs (e.g., UV-P, UV-326, UV-327, UV-328, and UV-329) have been detected in different marine compartments worldwide, such as marine waters and sediments. A variety of marine animals, from small crustaceans to large mammals, have accumulated BUVs. BUVs have been detected in aquatic environments at concentrations ranging from 0.05 to 99,200 ng/L; however, there are few studies on the effects of BUVs mixtures on aquatic organisms. Studies show that BUVs can affect behavioral responses and acetylcholinesterase activity, indicators of neurotoxicity. Additional studies support the hypothesis that BUVs induce oxidative stress and neuroinflammation in fish. The objective of this study was to evaluate the embryotoxicity of a mixture of BUVs and to use whole transcriptome sequencing to link toxicities with effects. We selected 5 BUVs including UV-326, UV-327, UV-328, UV-329, and UV-P for toxicological analysis, as these are among the most frequently detected in aquatic environments. Using microinjection, freshly fertilized embryos were exposed zebrafish embryos to 3 different doses of the BUVs mixtures before gastrulation, and effects on mortality, hatch success, heart rate, and malformations were evaluated until 14 days post-fertilization. We observed an increase in mortality dependent on the dose of BUVs, for the control group mortality was 2%, and 15, 23, and 33 % for the groups treated with BUVs. Likewise, BUVs caused a decrease in heart rate at 48 hours post-fertilization, the control group presented an average of 161 beats per minute (bpm), while the treated groups averaged 159, 157, and 156 bpm. Also, the larval size, eye area, yolk area, and melanophores in the head area in the treated groups decreased compared to the control group. Whole transcriptome (mRNA, non-coding RNAs) analysis is currently being performed to determine the molecular basis of the observed effects. This study provides new insight into the toxicity and hazards of aquatic organisms.

2.01.P-Mo120 Response of Freshwater Periphyton to Pesticides, their Mixture and Transformation Products: New Insight from Untargeted Metametabolomics

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Pesticides are widely used in agriculture and end up into aquatic environment where they may affect exposed organisms. All along this transfer process from field to river, pesticides can be degraded by abiotic processes and generate transformation products (TPs) for which the identity and the ecotoxicity is mostly unknown. Among these organisms, aquatic periphytons are complex assemblages of microorganisms with a short life cycle (e.g. microalgae, bacteria, etc.) playing a key role in aquatic ecosystems (e.g. primary production). Thus, they are relevant to investigate the impact of chemical contamination at the community level. Despite increasing knowledge on the impact of chemical stress, there is still a paucity of information about the effect of mixture of pesticides and their TPs on these communities. To such an end, metabolomics appears as a choice method by providing a comprehensive picture of the molecular phenotype. In this context, our study aims to evaluate the toxicity of the herbicide terbuthylazine (TBA) and its transformation products on periphyton through the implementation of a multi-descriptors approach. To do so, periphyton was exposed during 4 weeks to TBA, to one of its commercially available degradation product (TBA-desethyl), both at 5 and 150 µg/L and to weathered TBA (wTBA, homemade by photodegradation from TBA at 5 and 150 µg/L). To enhance the environmental realism, periphyton was exposed to TBA-desethyl in mixture with 2 ubiquitous contaminants, glyphosate and AMPA (GA) at environmental concentrations (0.1 and 0.3 µg/L, respectively). A control (without any contaminants) and GA conditions were also set-up to allow comparison between conditions. First, most descriptors (photosynthetic yield, biomass, heterotrophic enzymatic activities) were strongly affected by TBA and not or slightly by the transformation products, being the TBA-desethyl or the wTBA or the mixture with glyphosate. Conversely, multivariate analyses (PCA and HCA) showed discrepancies in the metabolomics profile between each condition, TBA 150 µg/L being the most discriminated while both 5 and 150 µg/L of TBA-desethyl and wTBA were also separated from the controls. Nevertheless, all conditions with GA had a similar metametabolome profile suggesting no mixture effect. Overall, this study confirms the higher sensitivity of the metametabolome to

micropollutants and so its relevance to highlight potential impact of TPs and mixtures on aquatic microbial communities.

2.01.P-Mo122 Screening of mixture toxicity of major biocides in *Allivibrio fischeri*

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In order to obtain authorisation for a biocidal product, it is necessary to conduct a mixture toxicity assessment of the active ingredients and co-formulations in biocidal products. Regarding the interactions between substances in mixtures, case-by-case testing is necessary, however, given the considerable variety and number of mixtures, appropriate toxicity data is rare. In this study, we selected a range of substances commonly used in wood preservatives and related biocidal products. Then we evaluated their mixture toxicity using the luminescent bacterium *Allivibrio fischeri*. In total, 26 individual biocides and 50 mixtures were selected for toxicity tests. The combinations most commonly used in wood preservatives were included in the study, as well as those ranked in order of toxicity. Two of the tested substances were found to act as synergists, while the majority of the mixtures followed a concentration addition (CA) model. In some cases, the synergistic effects were as high as 20-fold, indicating that appropriate assessment factors should be set when evaluating biocidal products or mixtures containing these substances. The results of this study need to be validated at a higher level in the future. This study was funded by National Institute of Chemical Safety through the Development of Environmental Risk Assessment Methodology of Biocidal mixtures, Korea Research Institute of Chemical Technology (KRICT) through the Development of Chemical Safety-by-Design Platform Technologies (Project No. KK2552-10), and Korea Environment Industry & Technology Institute (KEITI) through Technology Development Project for Safety Management of Household Chemical Products, funded by Korea Ministry of Environment (MOE) (Grant No. RS-2023-00215857).

2.01.P-Mo123 Development of Strategy for Mixture Toxicity Screening in Consumer Products: A Case Study on 1,2-Benzisothiazol-3(2H)-one

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Exposure to chemical mixtures through consumer products is a pervasive aspect of modern life, yet our understanding of the toxicity of such mixtures remains limited. In this study, we developed a strategy to identify chemical mixtures present in consumer products and to screen their potential systemic toxicity, focusing on 1,2-Benzisothiazol-3(2H)-one (BIT) as a case study. We gathered data, including the characteristics of components in products containing 1,2-Benzisothiazol-3(2H)-one (BIT), from Ecolife, a Korean consumer product database. To enable a component-based analysis, binary, ternary, and quaternary mixtures present in these products were identified. Mixtures for toxicity screening were selected with a focus on preservatives and chemicals known to induce allergic reactions. Equal-proportion mixtures were prepared and screened for cytotoxicity using four human cell lines (Beas-2b, E6-1, HepG2, and SH-SY5Y). Simultaneously, parameters derived from the dose-response curves of the individual chemicals constituting each mixture were applied to predict cytotoxicity of mixtures using the concentration addition (CA) model and the independent action (IA) model. The effects of the mixtures were assessed by comparing predicted values with experimental results through the model deviation ratio (MDR). For combinations exhibiting synergistic effects, the mixture ratios were varied to further verify whether the synergistic effects persisted across different compositional proportions. Ultimately, specific combinations of mixtures derived from consumer products containing BIT that demonstrated synergistic effects were identified. This case study demonstrates a strategy for identifying combinations of complex mixtures in consumer products that are likely to exhibit synergistic effects. These findings underscore the importance of accounting for mixture toxicity in consumer product risk assessments. Moreover, the proposed approach, incorporating in vitro screening, is expected to enable more efficient and comprehensive toxicological evaluations. This work was supported by Korea Environment Industry & Technology Institute (KEITI) through Technology Development Project for Safety Management of Household Chemical Products, funded by Korea Ministry of Environment (MOE)(RS-2023-00215309)

2.01.P-Mo124 Evaluation of Wastewater Treatment Plant Outfalls as Attractive Habitats for Fish and Invertebrates

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Wastewater treatment plant (WWTP) effluents are often loaded with pollutants that can cause harm to wildlife. However, these effluents can simultaneously host a seemingly advantageous environment with a

stable temperature, excess nutrients, and attractive neuroactive chemicals. Previous work in North America has shown that some species occur in greatest abundance near WWTP outfalls compared to up- and downstream sites, even though this may lead to greater uptake of contaminants. There is a need to understand if fish and invertebrates are attracted to these outfalls in Northern Europe, how these communities change along an effluent gradient, and how these patterns relate to chemical exposure. To this end, rivers around five Swedish WWTPs were sampled for fish using electrofishing and netting as well as invertebrates by kick-net sampling. Water and tissue samples were also taken to measure contaminant concentrations using an analytical suite of pharmaceuticals, personal care products, and pesticides. Preliminary results show that overall most fish were caught upstream of the WWTPs, however, there were species-specific differences of abundance with some species, such as burbot and tench, being more common near outfalls. Preliminary data from one WWTP shows that the total number of invertebrates were higher near the outfall compared to up- and downstream sites. These were largely highly pollutant tolerant taxa, such as chironomid midges and oligochaete worms. Here we show that certain species, especially those which are tolerant of pollution, appear to be attracted to WWTP outfalls. We will then combine these data with contaminant water and tissue concentrations to determine what is the exposure cost of inhabiting these areas. This research highlights the importance of understanding the effects of WWTP on receiving communities, which can help management strategies in these areas including whether tertiary treatment methods in WWTPs should be implemented. This work was funded by the Carl Trygger's Foundation and Swedish Research Council (FORMAS).

2.01.P-Mo125 Linking Aquatic-Terrestrial Ecosystems: Effects of Wastewater and Warming on Aquatic Insects

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Urbanization and wastewater discharges contribute to introduce complex mixtures of contaminants into freshwater ecosystems. These stressors in combination with the effects of climatic change (e.g. increasing water temperature), affect the ecological balance of aquatic ecosystems, and threaten their biodiversity. All these stressors influence each other and cross ecosystem boundaries through emerging aquatic insects. Emerging aquatic insects are key bioindicators in assessing how contaminants and climate change impact ecosystem health, as they are vectors of nutrients, contaminants, and energy across aquatic-terrestrial boundaries. Accordingly, the aim of the current study was to investigate single and combined effects of wastewater treatment plant effluent and increased water temperature on several community descriptors of aquatic insects. A two-month mesocosm experiment was conducted with a simplified freshwater food web containing aquatic bryophytes and insects, consisting of Ephemeroptera, Plecoptera and Trichoptera, feeding as shredders and grazers. Larvae and pupae were collected at the end of the experiment, whereas emerging insect taxa were collected daily. Changes in overall biomass, community survival, and emergence rates were analysed across different treatments. Additionally, analyses such as total lipid content and lipidome profiling are ongoing. The highest emergence rates were observed in the multiple stressors treatment (increased water temperature and presence of wastewater) and the increased temperature treatment, suggesting that temperature had a dominant influence on insect emergence. Although there were no statistically significant differences in community biomass across treatments, a trend of decreased biomass was observed for certain species exposed to increased temperature. Survival rates did not differ significantly between treatments. This study will provide novel insights into how aquatic insect communities respond to single and combined anthropogenic stressors while also outlining the implications for aquatic-terrestrial nutritional subsidies.

2.01.P-Mo126 Impact of Environmental Matrix Effects on Toxicity-driving Compounds in Wastewater Effluent

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Wastewater effluents contain micropollutants, such as pharmaceuticals and PFAS, posing risks to ecosystems and human health. Identifying key toxicity drivers is challenging due to environmental matrices (e.g., humic acids (HA), fulvic acids (FA), and a wealth of organic degradation products), which can mask or alter pollutant toxicity in concentrated samples, underestimating their impact at lower, more prolonged environmental exposures. This study used *Raphidocelis subcapitata* to assess the toxicity of wastewater effluents screened for over 300 target pollutants. In a subset of more than 250 samples, approximately 60 compounds were detected with an average detection frequency of 80%. By combining

EC50 values from algal growth inhibition tests with the quantified chemical data, we calculated toxic units (TUs) to evaluate the contribution of each compound to overall toxicity. In samples exhibiting toxicity, the biocide terbutryn and the pharmaceutical furosemide, explained 0.72%-143.66% of the total toxicity; however, over 50% of the toxicity remained unexplained in 154 samples. Notably, 23 samples that showed no detectable toxicity contained compounds at concentrations that should yield toxic effects ($TU > 1$), suggesting the potential for antagonistic effects or matrix interference. To further investigate the effects of matrix components on toxicity expression, we spiked azithromycin, melitracen, and terbutryn ($\log D$ at pH 7.4 = 0.78, 3.29 and 3.30, respectively) into two non-toxic wastewater samples with different chemical loads, testing at a concentration corresponding to a relative enrichment factor 30 in the highest concentration. The results showed that matrix effects had minimal impact on the toxicity of azithromycin, with only a slight increase in its EC50. In contrast, the matrices significantly suppressed the toxicity of melitracen and terbutryn, with melitracen's EC50 increasing by over ten-fold and terbutryn's by approximately three-fold, when added to the matrix. Absorption and fluorescence scans showed that dissolved organic matter (DOM) in wastewater, mainly HA and FA, suppressed pollutant toxicity, confirmed by spiking experiments with soil extracts. This study reveals the substantial impact of complex environmental matrices on toxicity expression. The fraction of unexplained toxicity may be underestimated, as bioassays with concentrated samples likely underrepresent toxicity in more dilute, bioavailable conditions.

2.01.P-Mo127 Assessing the Environmental Ecotoxicity of Wastewater Effluents Contaminated with Illicit Drugs and Benzodiazepines: Insights from Zebrafish Liver Cell Exposure

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The presence of emerging pollutants in aquatic environments has generated significant environmental health concerns, particularly with the increasing detection of pharmaceuticals and illicit drugs in surface and wastewater. Wastewater treatment plants are often unable to effectively eliminate these compounds leading to their continuous release into rivers, lakes, and oceans. This study aims to simulate real-world exposure conditions to assess the impact of illicit drugs and benzodiazepines on Zebrafish Liver Cells, which are the primary site of metabolism for many xenobiotics. The findings provide valuable insights into the potential risks these contaminants pose to aquatic life. This research focuses on the impacts on aquatic organisms and proposes a simpler, less costly method of analysis compared to Effect-Directed Analysis (EDA). To achieve this goal, two sample preparation methods were applied. The first method was based on Solid-Phase Extraction (SPE), and the second involved freeze-drying of the sample. SPE was employed to reduce sample complexity, enabling the establishment of the effects of illicit drugs and benzodiazepines on cells, as well as facilitating the pre-concentration of analytes. Freeze-drying provided a complementary approach to further concentrate contaminants without reducing sample complexity, allowing for a more accurate assessment of toxicity. Detection and quantification of illicit drugs and benzodiazepines with high sensitivity, even at trace levels, were conducted using HPLC-MS/MS. Meanwhile, cellular toxicity was determined using MTT and flow cytometry assays. The results indicate that the mixture of illicit drugs and benzodiazepines tested accounts for approximately 10% of the toxicity observed in the wastewater samples. This suggests that other compounds or synergistic effects between contaminants may also contribute to cytotoxicity. Additionally, the effect of filtration prior to cell exposure was studied and, in general, a decrease in sample toxicity was observed because of this process. In conclusion, these findings improve our understanding of the potential risks raised by the presence of benzodiazepines and illicit drugs in wastewater effluents. They highlight the ecological risks associated with these pollutants and emphasize the need to address this issue in the wastewater treatment processes. These insights can inform policy decisions and environmental management strategies aimed at protecting aquatic ecosystems.

2.01.P-Mo128 Effects of Urban Wastewater Effluents Containing Antibiotics Treated by an FBBR Reactor on the Aquatic Insect *Chironomus sancticaroli*

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Antibiotics are particularly significant among emerging contaminants because they can alter microbial communities, leading to increased antimicrobial resistance. Fluoroquinolones and sulfonamides represent two major classes of antibiotics that are widely utilized in human and veterinary medicine across the globe. This study assessed the ecotoxicological effects of effluent from an anaerobic fixed bed biofilm reactor (AnFBBR) that treats urban wastewater containing seven antibiotics: sulfamethoxazole, sulfadimethoxine, sulfamerazine, ciprofloxacin, pefloxacin, enrofloxacin, and ofloxacin. The evaluation focused on two phases of operation (control and spiked concentrations of antibiotics) and examined the

impact on a native Brazilian aquatic insect species, *Chironomus sancticarloi*, Strixino e Strixino, 1981. Acute ecotoxicity tests were conducted over a duration of 96 hours, during which organisms were exposed to five dilutions of the test solutions: influent I, influent II, effluent I, and effluent II, at concentrations of 60, 120, 250, 500, and 1000 mL/L. A control solution of demineralized water was also used. Since the species being tested spends most of its life cycle in the sediment, 50 grams of defaunated sand were added per triplicate. The results demonstrated that the treatment of urban wastewater effluent performed significantly better than the influent. A reduction in the toxicity of the effluents was observed in both phases across all concentrations tested. At the lowest concentrations (60 and 120 mL/L), the mortality rate was 6.6%. In contrast, the influent at a concentration of 1000 mL/L resulted in 100% mortality of the organisms, while the effluent at the same concentration showed an average mortality of 60%. In the Two-way ANOVA statistical analysis, the 60 mL/L dilution was found to be statistically equivalent to the control group (p-value = 0.1368). Additionally, the influents I and II displayed statistical similarity with a p-value of 0.3609. Based on the results obtained, we can conclude that ecotoxicity tests are valuable tools when combined with biomonitoring in reactors used for the degradation of emerging pollutants, such as antibiotics found in urban wastewater treated in anaerobic reactors. However, it is essential to implement complementary technologies alongside An FBBR to reduce the environmental impact of antibiotics, particularly concerning the spread and development of antimicrobial resistance genes.

2.01.P-Mo129 Application of Mixture Assessment Factor in South Korea's Chemical Regulation
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In Korea, risk assessment of chemicals is overseen by two major acts; 'Environmental Health Act' and 'Act on Registration and Evaluation of Chemical Substances' (K-REACH). 'Guideline on Procedures and Methods for Risk Assessment of Environmental Hazards' provides detailed methodology for conducting risk assessments for human health, ecology, and children's products for the execution of the laws. However, the methodology in which cumulative effects of mixtures is not thoroughly described. Therefore, in practice, risk assessment of mixtures is hardly conducted, and mixture risk assessment of unintentional mixtures in the environment has become one of the most pressing measures in which regulation must be implemented. Mixture assessment factor (MAF) is a tool developed to take into account the cumulative effects of mixtures in risk assessment. This concept allows for the risk assessment of multiple chemicals, and can allow the risk assessor to identify potential risk of the mixture even when the level of individual chemicals in the mixture is below the threshold for safety. Recently, the European Commission has proposed the incorporation of MAF into Europe's chemical regulatory framework, and is examining the potential value and utility of MAF in the context of EU REACH. In this study, we explored the possibility and the value of incorporating MAF in Korea's chemical regulation. We conducted literature search to identify studies that conducted risk assessment of mixtures in three different settings in Korea, and examined the data for the possibility of applying the MAF approach. Next, we conducted risk assessment using the MAF concept for examining the mixture effect of mixtures in the selected ecological studies. Our results demonstrate the utility of MAF in conducting ecological risk assessment of unintentional mixtures in the regulatory context, specifically the management of Korea's watersheds. This study was conducted with the generous support of KEITI (RS2024-002-15309).

2.01.P-Mo130 CheOSmix - A Data-Reuse Approach to Determining Chemical Mixture Risk Driver Heterogeneity in European Freshwater

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Chemical pollution of aquatic environments involves complex combinations of substances. Mixture exposure can provoke combined effects even if the concentrations of individual substances remain below their effect thresholds. Therefore, efforts are ongoing to better consider mixture effects in chemical safety regulation. A debate accompanies these efforts on whether only a few substances drive the aquatic mixture risk and, thus, whether more strictly regulating these individual substances would be sufficient or whether a large heterogeneous set of driver substances needs to be considered. Here, we employed a data-reuse strategy to investigate the heterogeneity of drivers of chemical mixture risk to aquatic species. We initially demonstrate that drivers are heterogeneous using data from a single measurement campaign that enables comparing drivers between sites due to a set of consistently measured substances. Subsequently, we extended our analysis to an extensive collection of chemical monitoring data from the NORMAN

network. These data originate from different measurement campaigns that focused on diverging sets of substances at various time points. We, therefore, aggregated the data quarterly and per quarter cluster sites according to the set of measured substances. Our study concludes that an extensive list of substances drives chemical mixture risk across Europe and that these substances were highly heterogeneous between locations. Notably, the drivers of mixture risk were species-specific and exhibited variability over time. Also, we established that lacking monitoring data hindered a more precise analysis, particularly regarding temporal variability. We have stored all collected and curated data in a graph database. We present a chatbot that builds on a graph database - grounded large-language AI model to translate between the researcher and our database and, therefore, allows querying this knowledge for non-computer science experts.

2.01.P-Mo131 The Use of Ecological Modelling in System-based Environmental Risk Assessment: Investigating the Influence of Landscape Heterogeneity and Pesticide Mixtures

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The current environmental risk assessment paradigm is limited to silos-based approaches that poorly represent the environmental complexity of pesticide exposure and effects and is in need of revision. To be able to assess the effects of chemicals in a holistic way, not only individual toxicity responses have to be linked to population responses, but also other factors influencing exposure, toxicity, and population dynamics and distribution should be considered. To support the shift towards such system-based approach, this research project uses mechanistic population models to investigate (i) the influence of landscape heterogeneity on populations of the non-target arthropod species *Orius laevigatus* and (ii) the impact of chemical mixtures on earthworm populations. The first part of the project takes an existing (unpublished) agent-based model in NetLogo as a basis, adding more ecological realism. The model will be translated into Python to better deal with the high computational demands of complex landscapes. The effects of landscape complexity on *O. laevigatus* population endpoints and the pest control ecosystem service will be investigated at two spatial scales: off-crop margins of variable age and structure of a single arable field in the UK, and multiple vineyards with surrounding areas (500m radius) along a gradient of increasing heterogeneity in Portugal. The environmental scenarios will be based on two case studies within the SYBERAC project, that will also provide collected sampling data with which the modelled population responses will be compared. The second part of the project focuses on the impact of exposure to pesticide mixtures on earthworm population endpoints and the provision of soil related ecosystem services, e.g., nutrient cycling, water regulation, and crop production. Spray application series of multiple pesticides will be included (with concentration addition assumptions) into the existing (open access) earthworm model and population responses will be compared to the sampling data collected within SYBERAC.

Additionally, the extent to which functional redundancy can be expected will be determined by comparing the sensitivity of *O. laevigatus* and earthworms to other service providing units observed in the case studies. The results of this project are expected to provide insights and tools required for the shift towards a more holistic environmental risk assessment in terrestrial ecosystems. This research is part of the SYBERAC project (www.syberac.eu) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 101135213.

2.01.P-Mo132 Modelling Effects of Multiple Stressors – A Case Study with Imidacloprid and Aquatic Insects

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Mayflies and other aquatic insects due to their high sensitivity to neonicotinoid insecticide exposure serve as important non-target indicators in environmental risk assessments. Acute toxicity testing primarily focuses on lethality; however, sublethal endpoints such as feeding, growth and reproduction are of additional importance when assessing toxicity at chronic timescales. Moreover, the interactions of chemical stressors with environmental stressors (e.g., temperature increase and food reduction) are underexplored. To enhance our understanding of the impact of neonicotinoid insecticides and of their interactions with environmental stressors on aquatic insects, a Dynamic Energy Budget (DEB) based toxicokinetic-toxicodynamic (TKTD) model was developed. The model was parameterized for two insect species, *Chironomus riparius* and *Cloeon dipterum*, based on experimental data on feeding inhibition and immobility. The models were subsequently used to explore the combined effects of multiple stressors, specifically, imidacloprid exposure, temperature and food availability, under constant and time variable exposure scenarios. Model simulations predict EC50 (50% effect concentration) values for mobility and food consumed, larval/nymphal duration, and survival to pupation/emergence. The timing of exposure

initiation during larval/nymphal development is a more significant factor compared to the duration of the duration of constant toxicant exposure, whereas under conditions of time-variable exposure, assuming an exponential decrease of the toxicant, the rate of decrease is more important than the timing of exposure. Temperature has a larger effect on larval/nymphal duration than the food availability, while survival to pupation/emergence was affected mostly by the toxicant concentration, with *C. riparius* exhibiting sharp responses to changes in toxicant concentration, whereas *C. dipterum* showing a more gradual effect. However, the EC50 value for mobility of *C. dipterum* is, by a factor 2, lower compared to the one of *C. riparius*, indicating the species greater sensitivity.

2.01.P-Mo133 Probabilistic Risk Characterisation for Chemical Mixtures: Hierarchical Integration of Models for Similar and Different Mode of Action

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Risk assessment for chemical mixtures has been subject to much research and method development in the last decade, but many challenges remain. Probabilistic risk methodologies are becoming more common in both human and environmental risk assessment (ERA), and can offer new approaches to fundamental challenges in ERA, such as the characterisation of mixtures risk. For pragmatic reasons, the Concentration Addition (CA) concept is regularly used in so-called deterministic risk assessment (e.g., sum of risk quotients). However, the Independent Action (IA) concept lends itself more easily to probabilistic risk calculation (e.g., joint probability of threshold exceedances). Here, we explore a combination of these two concepts in a hierarchical probabilistic model (object-oriented Bayesian network OOBN). First, probabilistic risk quotients (RQ) are calculated for individual chemicals, as probability distributions of environmental concentrations divided by a predicted no-effect concentration). Next, the CA concept is applied within groups of chemicals (e.g., with similar mode of action) by summing the RQ distributions. Finally, the IA concept is applied across the different chemical groups, to combine RQ distributions by joint probability calculation (OR expressions). This model is applied to case studies from the EU-funded project MULTISOURCE (ModULar Tools for Integrating enhanced natural treatment SOlutions in URban water CyclEs; <https://multisource.eu/>). This project is based on a set of seven technical pilots for improved wastewater treatment by nature-based solutions, across European countries and in USA. The aims of this poster presentation are: (1) to introduce a novel probabilistic approach to mixture risk characterisation, which integrates the CA and IA concepts in a hierarchical BN model; (2) to demonstrate the usefulness of the model to a real-life case study (a MULTISOURCE technical pilot) as a proof-of-concept; and (3) to evaluate the applicability of this approach for environmental risk assessment in more general contexts and at larger scales. The MULTISOURCE project has received funding from the European Union's Horizon H2020 innovation action programme under grant agreement 101003527.

2.01.P-Mo134 Bush Encroachment Coupled with Climate Change and Variability in Protected and Communal Areas: A Species Distribution Modelling Approach

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Savanna rangelands have experienced widespread degradation due to bush encroachment, raising significant concerns among conservationists and rural communities. In the context of climate change, these ecosystem shifts are likely to intensify, especially in South Africa's semi-arid regions. Understanding the impacts of climate variability and change on species distribution within these rangelands is crucial for mitigating further ecosystem disruption. Environmental factors, along with climatic variables, can accelerate the process of bush encroachment, threatening both biodiversity and land use. Early identification of areas vulnerable to invasion is key to developing effective and cost-efficient management strategies. This study aims to model the distribution of invasive species across protected and communal landscapes under long-term climate change projections. The current and future bioclimatic variables, as well as environmental and Sentinel-2 Multispectral Instrument satellite data, were used to fit the models and predict areas at risk of bush encroachment in the Kruger National Park and surrounding communal areas. The combined variables explained over 90% of the habitat suitability model under the current climate with Bio 2 being the most important variable (95%-53.5%) for RF and MaxEnt respectively. The future projections have also shown that slope, precipitation and temperature some of the key climatic variables were good predictors for the suitable areas for encroachment for both RCP 2.6 and RCP 8.5. Moreover, the overall predictions using the ensemble model demonstrated an increase in areas suitable for encroachment under RCP 8.5 but a decrease in the bush encroachment rate under RCP 2.6. These findings underscore the critical need for proactive management strategies to mitigate bush

encroachment, particularly under high-emission scenarios, ensuring the sustainability of semi-arid savanna rangelands in the face of climate change.

2.01.P-Mo135 Evaluation of the Population-level impacts of Chemical Exposures on Pacific Salmon in the Puget Sound, Washington, USA - Part 1 - Model Development and Legacy Contaminants

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Pacific salmon (*Oncorhynchus* spp.) are a culturally and economically important fish, found along the northwest coast of North America. They have a complex life history that includes stages in the freshwater, estuarine, and marine environments, with long migration routes. Population levels have declined markedly due to anthropogenic pressures including habitat loss, river dams, over exploitation, and exposures to chemical contaminants including polychlorinated biphenyls (PCBs). The effects of some of these contaminants have been well studied for individual fishes, yet this evaluation has not been widely applied to populations. The objective of this project was to understand the population level impacts of exposures to PCBs on different salmonid populations in the Puget Sound, WA, USA. To do so, we developed a set of life-history models, each representing a distinct population. These models provide the framework to understand the extent, duration, and range of chemical exposures, and to quantify the potential, population-level impacts. Chemical exposures in each life stage were estimated for populations, and some life stages, based on monitoring data collected in the region by the Washington Department of Fish and Wildlife. The effects of chemical exposures on population-relevant endpoints, including growth, survival, and reproduction were informed by published exposure-response relationships and/or developed from the literature. These effects were used to adjust the probability of survival of key life-stage transitions in the population model. The results indicated that there was a significant effect on the population specifically associated to chemical exposures, limiting the success of salmonid recovery and restoration activities. This highlights the need to improve our management of anthropogenic chemical loading to receiving waters. This population level effect was apparent based on an analysis of exposures to a single chemical group; environmental monitoring data indicates that salmonids are exposed to mixtures. Ongoing and future work will focus on developing and applying relationships for individual chemicals and mixtures.

2.01.P-Mo136 Probabilistic Modelling of Pharmaceutical Pollution in a Scottish Meso-scale Catchment

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Understanding how hydro-climatic interactions affect the movement of pollutant mixtures through the environment and how these interact with riverine ecosystems is a major research challenge. Riverine environments are affected by multiple combinations of physical, biological and chemical stressors. Although concentrations of some regulated pollutants have been reduced in recent decades, the increasing diversity of emerging contaminants of concern has resulted in a wide array of pollutant mixtures from a range of sources. These include wastewater treatment systems including combined sewer overflows (CSOs), rural septic tanks, agricultural and urban runoff and legacy pollutants from past practices. Simultaneously, climate driven shifts in water flows and temperatures are likely to influence how freshwater ecological communities function, with changes in exposure likely to magnify effects on biota. However, the combined effects of these pollutant mixtures and multiple stressors are poorly understood. Here, we develop a probabilistic model based on Bayesian Belief Networks to simulate contaminant inputs to the River Almond, Scotland, UK (369 km²), from its catchment. Pharmaceutical pollution from sewage treatment works (STWs), storm overflows, septic tanks and diffuse sources (veterinary prescribing, agricultural land use) will be simulated on a monthly time-step. Data on river flow, pharmaceutical prescription rates, the number, size and type of STWs, pharmaceutical-specific excretion rate and removal within STWs are integrated to simulate contaminant concentrations and loads at 20 sampling points. The model simulations for a set of selected pharmaceuticals showed reasonable agreement with measured concentrations. Ecological risk quotients (ERQs) using predicted environmental concentrations and no-observed-effect concentrations (NOEC), coupled with a probabilistic Precautionary Factor (as in Mentzel 2022), will be used to assess the impact of pollutant mixtures on stream macro-invertebrates, making the assumption that the stressor-response relationship is additive. A simplified version of the model will be upscaled to Scotland-wide to map pollution risk on an annual time-step to

identify pollution hot-spots and inform prioritisation of mitigation strategies. This research is funded by the UKRI Natural Environment Research Council Freshwater Quality Program (MOT4Rivers project), with in-kind contribution from the Scottish Government Strategic Research Program 2022-2027.

2.01.P-Mo137 Predicting Cellular Level Vulnerability to Combined Stress of a Non-Chemical Stressor and Chemical Toxicity Using the Stress Additional Model (SAM)

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In the environment, organisms face simultaneous chemical and non-chemical stressors. The Stress Addition Model (SAM) enables to predict the effect of combined stresses and has been applied to survival studies in vertebrates and *Daphnia*. Typically, non-chemical stress enhances chemical toxicity while reducing the steepness of dose-response curves. So far, studies on combined stress effects at the cellular level remain limited. This study investigates the impact of starvation (a non-chemical stress) combined with chemical stressors on the human lung cancer cell line A549. In this experiment, A549 cells were plated in 384-well plates in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum. After 24 hours, starvation was induced by replacing the medium with a mix of medium and phosphate-buffered saline (PBS) at 0%, 20%, 40%, 60%, 80%, or 100% PBS. Cells were then exposed to test chemicals for 24 hours: 3,4-dichloroaniline (nonspecific toxicant), staurosporine (a protein kinase inhibitor, inducing apoptosis), docetaxel (inhibitor of microtubule polymerization, disrupting proliferation), and saccharin (negative control). Three independent experiments were conducted with two technical replicates per condition and six concentrations per chemical. After 24 h of exposure, cells were live-labeled with Hoechst, Calcein-AM, and propidium iodide for 30 minutes, followed by fluorescent imaging. Viability was defined as calcein-positive cells relative to solvent controls, and cytotoxicity as propidium iodide-positive cells relative to total cells per well. Complete starvation resulted in significant cytotoxicity. In non-starved cells, staurosporine exhibited a steep concentration-response curve with a potency of 100 nM. However, in fully starved cells, the curve was less steep, with excess cytotoxicity observed at concentrations below 10 nM. In contrast, docetaxel induced cytotoxicity at concentrations above 10 nM in non-starved cells, but this effect was absent under starvation conditions. We hypothesize that under starvation, cells cease proliferating, thereby rendering docetaxel ineffective in inducing additional cell death.

In conclusion, these findings indicate that the Stress Addition Model (SAM) is applicable at the cellular level. However, its applicability appears to depend on the mode of action of the chemical stressor. A strong reduction in food was required to observe effects; consistent with studies in invertebrates and vertebrates.

2.01.P-Mo138 Linking Micropollutants Toxicity and Macroinvertebrate Communities in German Rivers Using AI-Based Toxicity Predictions

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Understanding the impact of chemical pollution on benthic invertebrate communities in streams and rivers is critical for assessing ecosystem health. However, linking chemical monitoring data with communities health remains challenging; chemical pollution rarely occurs alone but rather act in concert with other stressors, such as water scarcity or increasing temperatures, that might hinder that evaluation of chemicals toxic effects. Moreover, each individual compound has a its very own toxic effect that might be difficult to disentangled in the wide array of chemicals present in the system. In our study, we employed the recently developed AI-based ecotoxicological prediction model TRIDENT providing robust toxicity estimates for a wide range of micropollutants. We linked the chronic toxicity predictions of approximately 1100 compounds with monitoring data from German federal states at over 100 sampling sites. Using predicted EC10, we calculated different sets of toxic units (TUs) to estimate the overall toxicity of the water samples and the toxicity of several groups of micropollutants (e.g. pharmaceuticals, biocides). These estimates were subsequently integrated with data on benthic invertebrate community structures from sites near the location of the chemical samples. By linking chronic toxicity predictions with multimetric index and community assemblages, we derived valuable insights into the role of micropollutants in shaping benthic invertebrate communities. We also highlighted micropollutants with the most detrimental effects on the communities health. This approach not only enhances our ability to assess the ecological impacts of chemical pollution but also highlights the utility of AI-based methods in environmental monitoring and management. Our findings emphasize the need for integrating advanced predictive tools to better understand and mitigate the effects of micropollutants on aquatic ecosystems.

2.01.P-Mo140 Multistress in a Changing World: Impacts of Pollutant Mixture and Temperature on Gene Expression of the Freshwater Gastropod *Physella acuta*

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Aquatic ecosystems are highly vulnerable to climate change and pollution. Individual pollutants and combinations of anthropogenic compounds, including microplastics, pesticides, and heavy metals, impact these ecosystems. Diverse chemicals lead to complex interactions often overlooked while rising temperatures from climate change add stress to aquatic life. Due to the intricate nature of environmental conditions, typical toxicity assessments may not effectively capture the impacts and interactions of these factors that could affect aquatic invertebrates, ultimately putting their populations at risk. Therefore, to mimic closer environmental conditions, we exposed the freshwater gastropod *Physella acuta* to mixtures of three pollutants Bisphenol A (BPA), Endosulfan, and Cadmium. Exposure mimicked the warming scenario 3 proposed by IPCC (middle of the road: 2.7 °C by 2100), so control (18 °C) and warming scenario (20.5°C) were used. The animals were collected for gene expression analysis at 24 hours, 7 days, and 21 days. The effects were evaluated using retrotranscription and Real-Time PCR with genes related to stress response, endocrine system, and detoxification mechanisms. This study aims to collect data on temporal responses and elucidate the putative impact that global warming can have on the ability of aquatic invertebrates to respond to toxicity. The information gathered will also benefit other species, helping to know biomarkers that are helpful in this analysis. Changes in the genes are observed, and the results are discussed, considering the toxicants and temperature since both factors are relevant to the future of the populations. In addition, the experimental design will provide insights into the standardization of molecular studies for toxicity analysis in complex scenarios involving different factors that can be affected by the progress of global change. This work has been funded by Ministerio de Ciencia e Innovación (PID2022-136669OB-I00). A.M-B is a recipient of a fellowship from Ministerio de Ciencia e Innovación (PRE2022- 000781)

2.01.P-Mo141 Sublethal Chemical Exposure Alters Thermal Tolerances of Freshwater Fish and Invertebrates

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Chemicals not only exert direct effects on the growth, survival, and reproduction of organisms, but can also indirectly affect an organism's ability to confront shifting abiotic conditions, potentiating or moderating dire consequences in a multi-stressor world. Climate change, industrial effluents, and hydromorphological changes contribute to increasing temperatures, along with their fluctuations and extremes, in freshwater ecosystems. The thermal tolerance of freshwater organisms dictates their ability to handle these stressful temperatures. Chemical exposure can decrease the thermal tolerance of an organism by diverting energy towards detoxification processes or maintaining homeostasis, or increase thermal tolerance by activating a protective general stress response. Whether chemicals generally lead to higher or lower thermal tolerances in organisms, and how the concentration and characteristics of a chemical influence the effect, remains unknown.

To investigate the interaction between chemicals at sublethal concentrations and thermal tolerance, studies investigating thermal tolerance under chemical exposure were collated from the literature. The relationship between 15 substances, including heavy metals and organic chemicals, and thermal tolerances of freshwater fish and invertebrates (36 species-substance combinations) was compared. Most chemical exposures led to a reduction in thermal tolerance, with notable exceptions. Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) SFB 1439/1 2021 426547801

2.01.P-Mo142 How Does Copper Alter the Thermal Tolerance of *Calanus* Copepods in Oslo Fjord?

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Fjord ecosystems are commonly found in Arctic and subarctic regions. These unique landscapes feature long, narrow sea inlets with steep cliffs or slopes, shaped by glacial activity. Their distinctive morphology and strategic locations have historically made fjords hubs for human settlements. However, they are increasingly affected by climate change and human activities, including shipping and aquaculture. This is particularly true for Norwegian fjords such as Oslofjord where both warming and pollutants such as copper have been major issues for key fjord species such as *Calanus* copepods. Copper and warming have been known to interact synergistically to intensify their cumulative effects on exposed copepods.

Furthermore, pronounced seasonal changes in the fjord environmental conditions and physiology of *Calanus* copepods in Oslofjord may affect their sensitivity to warming and copper, but this remains to be tested. The objective of this study is to disentangle the role of seasonality with two contrasting seasons:

winter and summer on the vulnerability of *Calanus* copepods in the Oslofjord to copper and elevated temperatures. Here we exposed *Calanus* copepods collected from Oslofjord to 5 temperatures (5, 10, 15, 20 and 25°C) in combination with 4 different copper concentrations (0, 20, 40, 60 and 120 µg/L). The daily survival was then monitored over 4 days. Lipid sack and size were measured as an indication of energy consumption during the exposure. To explore the genotoxic effects of warming and copper, we analyzed DNA damage of surviving individuals. We are analyzing the data and will provide the main findings at the conference. Results of this study might provide mechanistic insights into the vulnerability of *Calanus* copepods to warming and pollutants with applications for ecological risk assessments of fjord ecosystems.

2.01.P-Mo143 Comparing the Thermal Tipping Point of the Overwintering and Summer Arctic *Calanus* Copepods in Response to Copper Pollution

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One of the main threats for arctic species is ocean warming, which is approximately 4 times faster than the other seas and oceans. The Arctic warming is intensified by the influence of the warm Atlantic water, particularly during the winter while several Arctic zooplankton are overwintering. In the summer, a great concern is marine heatwaves, i.e. sudden peak in maximal temperatures, that can last several weeks and should increase in intensity, duration and frequency. It is unknown if the Arctic warming may approach the thermal tipping points (TTPs) of Arctic copepods, a tolerance threshold beyond which the survival copepods is impacted is primordial to project on their fate. Furthermore, Arctic zooplankton are increasingly exposed to pollutants from human activities such as copper from increasing shipping and aquaculture. Copper exposure has been known reduce the tolerance of polar copepod to warming, therefore, it is predicted to lower their TTPs. It has never been tested 1) whether exposure to copper may reduce TTP of the dominant Arctic copepods from the genus *Calanus* and, if yes, 2) how changes in TTP of *Calanus* copepod differ between winter and summer due to differences in the copepod physiology. The objective of this study is to determine the effect of copper on the survival of copepods at elevated temperatures by assessing survival over a wide range of temperatures and estimating thermal tolerance threshold, with a focus on how seasonality and extreme winter conditions may influence these effects. Sixteen individuals were individually placed in 50 ml flasks with different copper conditions (0, 20, 60, 120 µg/L) at different temperatures (0°C, 5°C, 10°C, 15°C, 20 °C) in incubators. The survival was checked daily over four and six days. Survival was modelled as a function of temperature and copper conditions using Cox proportional-hazards model, and Kaplan-Meier survival curves. Preliminary analyses showed that the tolerance threshold for copper was identified between 0 and 20 µg/L, while the thermal tipping point was found between 15°C and 20 °C. Above 20 µg/L, the effect of copper dominated and masked the effect of temperature on survival. Seasonality did not seem to affect the sensitivity to temperature and copper.

2.01.P-Mo144 Chronic Toxicity of Methoxyfenozide to Aquatic Macroinvertebrates at Two Different Temperatures

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Methoxyfenozide, a diacylhydrazine insect growth regulator, has become a globally popular insecticide due to its high specificity for lepidopteran pests. As an ecdysone agonist, methoxyfenozide mimics the molting hormone and triggers premature molting, leading to developmental arrest and mortality. However, its environmental persistence and potential to disrupt endocrine systems in non-target organisms raise concerns. Furthermore, temperature an environmental stressor amplified by climate change can modulate pesticide toxicity, potentially increasing its risks to aquatic ecosystems. The acute toxicity of methoxyfenozide has been studied in *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles gambia* for 10 day period and in *Chironomus tentans* for 5 days in the endpoint point of mortality. Moreover, a developmental study highlighted the significant potency of methoxyfenozide against *C.tentans*, showing activity comparable to its high efficacy observed in lepidopteran larvae. In this study, we evaluate the chronic toxicity of methoxyfenozide at different temperatures to bridge a critical knowledge gap in understanding the ecological risk of long-term effects of methoxyfenozide, especially under climate change. We tested insect and crustacea as the models because they have homologous endocrine pathways. We exposed *Gammarus pulex* and *Cloeon dipterum* juveniles to methoxyfenozide in 7 concentrations ranging from 0 to 100 µg/L under 10 and 18 °C. We assessed survival, immobility, and food consumption during the chronic test of 28 days. Results revealed that the 28d, EC50 of *C.dipterum* at 10 and 18 °C were 126 and 75 µg/L, respectively, indicating an increased toxicity at higher temperatures. The 28d, EC50 of *G.pulex* were much higher than the highest concentration tested. *C.dipterum* showed a lower EC50 than

G. pulex, consistent with the mode action of methoxyfenozide. Survival probability in all treatments was lower with higher temperatures. These findings emphasize the need for integrated assessments considering multiple stressors. Follow-up experiments will investigate mechanistic processes by including bioaccumulation and multi-generation to evaluate cumulative long-term effects on non-target species, especially insect larvae. These studies would enhance understanding of the long-term impact of methoxyfenozide for guiding sustainable pesticide use.

2.01.P-Mo145 Impact of Increasing Temperature Fluctuations on the Toxicity of λ -Cyhalothrin to *Chironomus riparius*

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Ongoing global warming and the associated increase in water temperature will have a variety of effects on freshwater habitats. The threat posed to non-target organisms by plant protection products may increase as a result. Temperature is an important factor in ecotoxicological studies, it acts as a stress factor and can influence the physiological state of organisms. Rising average temperatures can lower toxicological endpoints of risk assessment. In addition to average temperatures, the intensity and frequency of daily temperature fluctuations will also increase in the future. Ecotoxicological studies have already shown that the increase in daily temperature fluctuations has a greater impact on organisms than the increase in the average temperature. This might be crucial for evaluating the toxicity of plant protection products and thus could play a relevant role in future risk assessment. Our aim is to record the effects of plant protection products on non-target organisms in the event of (increasing) daily temperature fluctuations. We present data of acute ecotoxicological studies with *Chironomus riparius* and λ -Cyhalothrin (according to OECD 235) with three different temperature scenarios where the daily fluctuation is either 0, ± 5 or $\pm 10^\circ\text{C}$.

2.01.P-Mo146 Influences of Sertraline on the Activity Patterns of the Phantom Midge Larvae *Chaoborus* Under Varying Temperature Conditions

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Global change significantly impacts freshwater ecosystems, contributing to biodiversity loss and ecosystem instability. Among various anthropogenic influences, rising temperatures and micropollutants, such as antidepressants, are major stressors. Selective serotonin reuptake inhibitors (SSRIs), like sertraline, are increasingly found in freshwater due to rising prescription rates and low removal efficiency in wastewater treatment plants. These neuroactive substances affect serotonergic pathways in invertebrates, often linked to feeding behavior and activity patterns. Simultaneously, elevated temperatures accelerate metabolic rates, potentially altering behavior and interaction dynamics in freshwater organisms. Behavioral adaptations are critical for survival in the context of predation. Prey often reduce activity to avoid detection, while ambush predators, like the phantom midge *Chaoborus*, rely on motionless stealth to capture prey. These strategies may be compromised under stress from elevated temperatures or exposure to neurostimulants. Furthermore, *Chaoborus*, a key species in freshwater food webs, preys on zooplankton and serves as prey for fish, transferring energy up the food chain. Stressor-induced behavioral changes, such as reduced predation rates on zooplankton and increased vulnerability to fish predation, could destabilize these ecosystems. In this study, we investigated the effects of sertraline and elevated temperatures on *Chaoborus* larvae activity and predation patterns. Larvae were exposed to sertraline under ambient and elevated temperature conditions. Results showed that both stressors reduced overall activity and predation rates. Reduced predation by *Chaoborus* could lead to increased zooplankton populations, such as *Daphnia*, altering community composition and improving water clarity through enhanced phytoplankton grazing. These changes may disrupt trophic dynamics, reducing energy transfer to higher predators, such as fish, and shifting nutrient cycling processes. Understanding how combined stressors impact predator-prey dynamics is crucial for predicting and mitigating global change effects on aquatic ecosystems. This study highlights the importance of addressing multiple anthropogenic pressures to preserve freshwater ecosystem health and functionality.

2.01.P-Mo147 Interactive effects of mixtures of Copper and Pendimethalin to Microalgae – Temperature Matters!

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Climate changes are linked to extreme weather events affecting various activities and ecosystems. The growing world population and food consumption demand intensive use of fertilizers and pesticides. Among pesticides, copper and pendimethalin have been commonly used. Copper is widely used in biological agriculture and is component of several other pesticides. Although it is an essential metal, it becomes toxic in high amounts. Pendimethalin is a widely used herbicide of the dinitroaniline family, used to control annual grass and broadleaf weeds in various crops. When these contaminants enter in aquatic systems, they can harm non-target organisms like microalgae, which are similar to other plants and become unintended targets of herbicides. Therefore, it's crucial to determine if climate changes influence the combined toxicity of pesticides on microalgae. This study evaluates how temperature affects the toxicity of copper and pendimethalin mixtures on microalgae, using *Raphidocelis subcapitata* as a model, at environmentally realistic concentrations. Growth inhibition assays were performed at 15°C, 20 °C and 25°C, and the obtained yield data were modelled using the mixture models Concentration Addition (CA) and Independent Action (IA), accounting for possible deviations. Both models fit the experimental data similarly, with the IA model performing slightly better at 15°C and 25°C. Considering the distinct modes of action of both compounds, the IA model was chosen to describe and predict the interactive effects of both contaminants. At 15°C, the baseline IA model described better the obtained data ($R^2 = 0.8837$). At 20°C, the model that best described the data was IA with a Dose Ratio deviation ($R^2 = 0.9345$), showing antagonistic effects, most likely caused by copper. At 25°C, the IA model with a Dose Level deviation was the model which best explained the data ($R^2 = 0.9544$), showing antagonistic effects at low concentrations but predicting synergetic effects at higher concentrations. These results indicate that rising temperatures could alter the interactive effects of both contaminants on freshwater microalgae. At higher temperatures, there was a trend for antagonistic interactions at environmentally realistic concentrations, suggesting that the combined toxicity of these contaminants might be lower than predicted based on their individual toxicity at low temperatures. These findings enhance our understanding of climate change's effects on microalgae.

2.01.P-Mo148 Effects of Altered Metamorphosis on the Population Maintenance of Amphibians - A Systematic Review

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The Regulation 2018/605 laying down the scientific criteria for the identification of pesticides having endocrine disrupting (ED) properties on humans and non-target organisms was published in 2018. To reach a conclusion on ED properties for wild species according this regulation, amphibians are considered the model species based on the available knowledge and test methodology. For amphibians, any effect on metamorphosis is considered adverse. However, a better understanding of the extent to which metamorphosis have to be altered to produce an impairment on populations is needed. Protocols and search strategies were developed for this project. Consequently, an extensive review of the literature was done to identify effects of altered metamorphosis on the population maintenance of amphibians. For the systematic review a search strategy was developed to identification of 2342 papers based on a research question. The relevance analysis of the documents according to the eligibility criteria did not identify any articles which would answer the initial question. Nevertheless, articles focused on metamorphosis change or population strategies of various amphibians were identified. Findings showed the complexity of populations, interactions and complexity of environmental interactions and various species life and reproduction strategies of orders Anura and Urodela. Based on this review a research which investigates effects on metamorphosis assessed in AMA and simultaneously investigate consequence of such effects on population maintenance does not exist. However, there are several technics used in the ecology research present in examined studies which could allow to conduct future population study for linking the laboratory experiments such as AMA with the in situ population research. Question in such study would be the extra species extrapolation as species use different life strategies, but at least as a proof of concept of metamorphosis effects on population maintenance a study could be designed.

2.01.P-Mo149 Oxidative Status In Two Populations of the Invasive Asian Clam in a Major Estuary in South America

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Invasive species face multiple environmental stressors within the ecosystems they colonize. In aquatic habitats, these stressors include pollution, parasites, and variations in water temperature, which can significantly affect the competitive abilities of these species and potentially restrict their expansion. The present study evaluated two populations of the invasive clam *Corbicula fluminea* in the Río de la Plata estuary, one of Argentina's most significant waterbodies. These populations are exposed to varying levels of anthropogenic impact: Parque de los Niños (PN), in the northern part of the estuary, is heavily impacted by urban pollution, whereas Punta Lara (PL), situated 74 km to the south, is away from this influence. Between October and November 2023, we collected clams and water samples from both sites. Live clams were transported to the laboratory to assess their gill oxidative status and examine the potential influence of the gill parasite *Chaetogaster limnaei* at two different temperatures: 21°C (the water temperature at the time of sampling) and 12°C (the average water temperature during the winter). The infection prevalences were also determined. Oxidative stress biomarkers analyzed included the antioxidant activities of reduced glutathione (GSH), glutathione S-transferase (GST), and catalase (CAT), as well as thiobarbituric acid reactive substances (TBARS), as indicator of lipid peroxidation. Metal concentrations (Cd, Co, Cr, Cu, Fe, Ni, Pb, and Zn) were analyzed in the water. Significant differences ($P < 0.05$) in cobalt (Co), iron (Fe), and lead (Pb) were observed between the two sites, with higher levels detected in PN. Parasite prevalence was 6% and 4% in PL and 43% and 40% in PN at 12°C and 21°C, respectively. While temperature and parasite prevalence significantly affect all the studied variables (CAT, GST, GSH, and TBARS) of clams collected at PN, these two factors only affect GSH and TBARS activities of the studied clams at PL. These findings suggest differences in the oxidative stress status of the two *C. fluminea* populations, possibly influenced by the biotic and abiotic conditions of the two sites in the Río de la Plata estuary. Clams from PL demonstrated potentially higher fitness, characterized by lower infection prevalence and reduced metal concentrations. These factors may play a role in determining the survival and spread of this highly invasive species to novel habitats.

2.01.P-Mo150 Evaluation of the Population-Level Impacts of Chemical Exposures on Pacific Salmon in the Puget Sound, Washington, USA. - Part 2 – Emerging Contaminants and Sublethal Effects

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In the western United States, several wild Chinook salmon (*Oncorhynchus tshawytscha*) populations have been listed as either threatened or endangered under the U.S. Endangered Species Act. Declining populations have been attributed to salmon habitat quantity and quality, including the presence of anthropogenic contaminants. Chinook salmon are particularly susceptible to contaminant exposures during critical early life stages spent rearing and foraging in rivers and estuaries where contaminants enter the environment from multiple sources. Polybrominated diphenyl ethers (PBDEs) are a group of contaminants that have been widely used as flame retardants in consumer products such as electronics, textiles, and plastics. While the production of many PBDE mixtures has been phased out due to human health concerns, certain congeners, including BDE-47 and BDE-99, remain prevalent, and continue to be detected in Chinook salmon tissue in Puget Sound, WA, USA. Despite emerging concerns regarding the adverse effects of PBDE exposure on individual salmon health, their population level impacts have not been investigated. This study aims to evaluate the impacts of PBDE exposure on Chinook salmon populations, focusing on the interactive effects of PBDE mixtures, particularly BDE-47 and BDE-99, on host-susceptibility to disease. Using the population model developed for legacy chemicals with field-collected PBDE exposure data and *Listonella anguillarum* as a model pathogen, this work estimates the incidence of delayed disease-induced mortalities in juvenile Chinook salmon populations in Puget Sound. This work improves our understanding of how sublethal effects, such as immunosuppression, contribute to population-level impacts, underscoring the potential benefits of pollution remediation efforts in promoting salmon recovery in the Pacific Northwest.

2.01.P-Mo151 Chemical Stressors and Their Impacts on Edaphic Organisms: The Role of MPs and Pesticides

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Terrestrial ecosystems are increasingly subject to simultaneous exposure to multiple chemical stressors, such as pesticides and microplastics (MPs), whose interactions can cause impacts are still unknown. MPs, due to their high persistence, can adsorb pesticides and release plastic additives, altering the degradation,

transport, and ecotoxicity patterns of these compounds in the soil. In countries with a strong presence of agribusiness, such as Brazil, MPs from agricultural plastic materials are one of the main sources of this type of pollutant, contributing significantly to the contamination of various ecosystems. In this regard, this study aims to investigate the ecological and toxicological impacts of the interaction between polyethylene microparticles and the pesticides imidacloprid and tebuconazole in natural soil, considering the coexistence and interaction of these contaminants. Experiments include conventional level I ecotoxicological tests and intermediate multispecies systems, focusing on survival, reproduction, biochemical changes, and changes in soil community composition. The edaphic species used include *Sinella curviseta*, *Proisotoma minuta*, and *Enchytraeus crypticus*, while parallel trials with *Eruca sativa* and *Allium cepa* will evaluate the effects on plants. The experimental design reflects environmentally relevant conditions, such as precipitation simulations and repeated applications of contaminants, to investigate the cumulative effects of these residues and their impacts on soil organisms. We start from the hypothesis that the interaction between MPs and the soil alters the bioavailability of MPs to organisms, interfering with the fate and toxicity of pesticides. Furthermore, toxicity to edaphic species may vary according to their position in the soil layers (euedaphic and epedaphic). This study is expected to provide unprecedented data on the interactions between MPs and pesticides, contributing to a more realistic and in-depth risk analysis. To our knowledge, this study is the first to address the toxicity of MPs associated with pesticides in multispecies systems, significantly expanding knowledge about the impacts on soil organisms. Therefore, the study can support public environmental management policies and effective strategies for mitigating ecological risks in tropical soils. A.P.C.A. thanks the Laboratory of Ecotoxicology and Applied Ecology EESC/USP (University of São Paulo) for the research opportunity and wants to express her gratefulness to the São Paulo State Research Foundation (process: 2024/12350-0).

2.01.P-Mo152 Ecotoxicological Assessment of Anti-Tuberculosis Medications in Aquatic Environments: Occurrence, Reproductive Effects and Ecological Risks

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Antituberculosis medications have the potential to elicit a range of adverse effects, such as neurotoxicity, and reproductive toxicity, in terrestrial organisms. Limited knowledge exists about the potential consequences of chronic exposure in aquatic ecosystems. This study aims to assess the reproductive toxicity of isoniazid (INH), rifampicin (RMP), and ethambutol (EMB) and exposure risk in aquatic organisms. We conducted reproduction tests on *Daphnia magna*, and measured residues of antituberculosis medication in aquatic environments to assess the ecotoxicological risks. Except for EMB, INH and RMP exposures did not affect number of broods and did not delay the time to the first brood at high concentration exposures in daphnia. However, three antituberculosis medications caused a significant decrease in the total number of neonates. We used the Benchmark dose to determine the thresholds for 2.80 mg/L of INH, 1.37 mg/L of RMP, and 22.14 mg/L of EMB in aquatic environments. For environmental residues, EMP has the highest frequency detection of 92% in rivers, while INH has the highest environmental residue level ranging from 81.01 to 547.88 ng/L, with RMP shows the lowest residue level and detection rate in rivers. Although there are no ecotoxicological risk concerns, INH has the highest exposure risk with a risk quotient of 0.03 (95%CI: 0.0018 0.1630). Here, these findings contribute to the ecotoxicological impacts of antituberculosis medications in aquatic environments and highlights isoniazid as a potential concern due to its highest residues and exposure risk.

2.01.P-Mo153 The Practical Application of Toxic Pressure and Bioassays for the Micropollutants Measurement Network HHNK 2023

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Hoogheemraadschap Hollands Noorderkwartier (HHNK) has been investigating the topic of micropollutants since 2014. The micropollutant monitoring aims to gain insight into the situation in our water management area and to develop knowledge about the role of sewage treatment plants (WWTP) in the removal and transport of pharmaceutical residues. The Key Factor Toxicity uses several methods to determine the toxicity of substances. One of them is to calculate the toxic pressure via the species sensitivity distribution (SSD) per substance. From these SSD curves the percentage of potentially affected fraction (PAF) per concentration of the substance can be read. When this is done for each substance, all PAF results can be added together to arrive at a more substances (ms)PAF. In addition to calculating toxic pressure (TP) the response of living organisms can also be looked at through bioassays. HHNK sampled effluent from WWTPs and surface water taken in June and September of 2023. The following bioassays were conducted: *Daphniatox*, *Microtox*, PAH-Calux, ER-Calux, PXR-Calux, and GR-Calux, P53-Calux (-S9). Overall, September scores can be seen to be somewhat worse than June. The GR-CALUX and the

P53-CALUX score relatively well. In contrast, the PXR-CALUX stands out as it scores poorly in almost all samples. This bioassay measures toxicant conversion and is sensitive to a wide range of substances. The Microtox, PAH-Calux and ERe-Calux score moderately. In all bioassays, more frequent and/or higher effects were observed in effluent than in surface water. The TP calculations give a good picture for the selected list of substances. However, this underestimates the actual toxic pressure. Not all substances are measured and included in the TP even though they may be present in the sample, and the SSD curves are also not available for all substances. The results of the TP and bioassays are broadly similar. For example, both results show that higher effect and toxic pressures were observed in effluent than in surface water. HHNK recommends continuing monitoring this monitoring network and annually update the analysis with the via data. This will provide insight into trends but also give a better picture of the toxic effects and pressures within the area. As more and more substances come onto the market, it will eventually become impossible to monitor them all through chemical analysis. Bioassays can be a valuable addition to that. HHNK is a Dutch government agency.

2.01.P-Mo154 Assessing the Reproductive Status of Fathead Minnows (*Pimephales promelas*) Exposed to Frank Lake, AB, Canada Effluent as a Bio-indicator of Ecosystem Health

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Municipal and industrial wastewaters are complex chemical cocktails, including personal-care products, pharmaceuticals, and pesticides, many of which are endocrine disrupting chemicals. Frank Lake (FL) is a multi-basin wetland in southern Alberta, Canada, that was restored to stable water levels in 1990 with the addition of a combined effluent from the town of High River and the Cargill beef processing facility. At high precipitation levels, water from FL drains into the Little Bow River that ultimately drains into the Twin Valley Reservoir, Alberta's newest reservoir. The biological effects that input of largely uncharacterized chemicals might be causing in the wetland, river, and multi-use reservoir are unknown. The study objective was to assess the reproductive physiology of fathead minnows exposed to the combined effluent from High River and Cargill. Standard 96-hr and 21-day assays were performed to assess reproductive toxicity, with the 96-hr assays being completed three times over the summer season of 2024 to quantify temporal variation in effluent toxicity. Fecundity and health indices (female condition factor, gonadosomatic index, hepatosomatic index) were not affected with exposure. Males exposed to high (75%) effluent concentration had a 25% decrease in condition factor ($p=0.0441$) and a 16% decrease in fertilization success ($p=0.0247$). Additionally, the tubercle score of males was increased by 55% with high effluent concentration ($p=0.0068$). Plasma concentrations of sex steroids and vitellogenin, expression of genes along the hypothalamus-pituitary-gonad-liver axis, and histological assessment of testes are being used to determine molecular and biochemical mechanisms of reproductive impairment in male fish. To complement laboratory assays, the same analyses of plasma and tissues are being assessed in male and female fathead minnows captured from Frank Lake and from a reference lake that does not receive effluent. This research will expand the understanding of industrial and municipal effluent impact on aquatic ecosystems, including health of exposed biota, and inform future management decisions regarding Frank Lake.

2.01.P-Mo155 Co-occurrence of Microplastics and Contaminants of Emerging Concern in River Water, Sediment and Fish: The Case Study of Júcar River Basin (Spain)

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There is rising concern about the environmental pollution by microplastics (MPs), especially about their ability to accumulate various types of contaminants on their surface. These contaminants include pesticides, pharmaceutical and personal care products (PPCPs), per-/ poly-fluoroalkyl substances (PFASs) and polycyclic aromatic hydrocarbons (PAHs). The co-occurrence and combined toxicity of MPs and organic pollutants needs to be studied. Thus, the presence of MPs, pesticides, PFASs, PPCPs and PAHs in water, sediment and fish samples from the Júcar River basin (Spain) was determined. The Júcar River flows through three different provinces, being one of the most important rivers in E Spain. The extraction was optimized for each matrix and contaminant. Density separation and digestion were combined for MPs extraction from water and sediment, while a microwave-assisted digestion was performed for MPs present in the fish gastrointestinal track. For the organic pollutants, solid-phase extraction and microwave-assisted extraction were adapted for each matrix. For MPs, ?FTIR and Pyrolysis coupled with Gas Chromatography-Mass Spectrometry (Py-GC-MS) were utilized, while the PAHs were analysed using GC-MS and the rest of the contaminants were analyzed by High Pressure Liquid Chromatography-High

Resolution Mass Spectrometry (HPLC-HRMS/MS), in both target and non-target modes. ?FTIR indicated the presence of MPs in all water samples (0.1- 6.9 MPs L⁻¹), whereas 73% of the sediment samples and 66% of the fish samples were polluted by MPs (sediments: 20- 140 MPs Kg⁻¹, fish: 6.7- 26.7 MPs g⁻¹). The main polymer types were polyethylene (water and sediments) and polyvinyl chloride (fish). Tebuconazole and difenoconazole were the most abundant pesticides in water. Perfluorobutane sulfonate and the painkillers ibuprofen and tramadol, were found in all water samples. The initial water analyses points to correlations between MPs and pesticides (i.e. propazine, imazalil, thiabendazole) and the flame retardant tris(chloropropyl) phosphate, particularly at two sampling points (highest MP amounts). Completing all analyses of sediment and fish samples will provide a better understanding of MPs and emerging contaminants co-occurrence. The risk assessment that is currently being performed, allows the estimation of the environmental toxicity that multiple anthropogenic pressures cause to wildlife and human health in a basin representative of the Mediterranean. This work has been supported by the Generalitat Valenciana (GV) through the Prometeo Programme CIPROM/2021/032. V. Soursoy also acknowledges her pre-doctoral contract ACIF/2021/408 and the grant CIBEFP/2023/154, both funded by the GV. The latter allowed her to work at the University of Pisa, Italy for 3 months.

2.01.P-Mo156 Vulnerability of Groundwater Species to Wildfire Ashes

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Subterranean ecosystems possess unique characteristics and host specialized organisms critical for maintaining groundwater quality. Despite their ecological stability, cave habitats are increasingly threatened by contamination from anthropogenic and natural sources, including wildfires. Wildfires are diffuse sources of water pollution, impacting surface and groundwater systems. Wildfire ashes contain harmful organic and inorganic compounds, such as polycyclic aromatic hydrocarbons and metals, which pose significant environmental risks due to their toxicity and bioaccumulation potential. This study assessed, for the first time, the effects of wildfire-related compounds on two groundwater-adapted crustaceans (stygobionts), *Proasellus lusitanicus* and *P. assaforensis* (Isopoda: Asellidae), differing in their adaptation to subterranean habitats. Organisms were exposed to ash dilutions, followed by biomarker analyses targeting detoxification, antioxidant defenses, oxidative damage, metabolism, and neurophysiology. Biomarkers included glutathione S-transferase, total glutathione, catalase, lipid peroxidation, electron transport system activity, lactate dehydrogenase, acetylcholinesterase, and total protein. Results revealed elevated lipid peroxidation in both species, indicating oxidative stress. *P. assaforensis*, the less groundwater-adapted species, showed increased lactate dehydrogenase activity at 1.25 g/L ash concentration, producing sufficient energy to counteract oxidative damage. However, this mechanism failed at higher concentrations. Conversely, *P. lusitanicus*, the more specialized species, experienced significant catalase inhibition across all tested concentrations and heightened ventilatory activity at the highest concentrations. This response suggests energy-seeking behavior, as *P. lusitanicus* lacks anaerobic energy production capacity, likely due to its adaptation to the stable subterranean environment. This specialization makes *P. lusitanicus* more vulnerable to stressors compared to *P. assaforensis*. These findings highlight the susceptibility of groundwater communities to wildfire-derived stressors, potentially disrupting ecosystem functioning and water quality. The loss of these species could impair subterranean ecosystems, triggering cascading ecological imbalances and affecting the water quality. Preventive conservation measures are critical to protect these organisms and preserve the ecological integrity of subterranean habitats.

2.02 Evolutionary and Mechanistic Insights to Improve Ecotoxicology

2.02.T-01 The Use of Ecological Relevant High-Throughput Video-Tracking Behavioral Responses in *Daphnia magna* in Environmental Risk Assessment of Chemicals

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Recent advances in imaging allow to monitor in real time the behaviour of individuals under a given stress. Light is a common stressor that alters the behaviour of fish larvae and many aquatic invertebrate species. The water flea *Daphnia magna* as many other zooplanktonic species show anti-predatory behavioural responses to light to avoid visual predators like fish. Antipredator behavioural responses include escape responses to light such as increase motility under light and diel vertical migration characterized by swimming towards deep and dark waters during daylight and towards surface waters during night to feed on alga. It is also known that many of those species used particular light wavelengths to trigger their migratory movements and other ones to search for food. The aim of this talk is to show recent developments using commercial and custom made high-throughput video-tracking devices that

have been used to study how neuro-active chemicals affect cognitive responses phylogenetically conserved such as those belonging to short term non associative learning to repetitive stimuli (sensitization, habituation), visual motor responses and phototaxis including colour preference. We also provide omic data anchoring those responses with known neurological molecular pathways. Examples include how illicit drugs such as cocaine, methamphetamine, MDMA and ketamine disrupt serotonergic and dopaminergic signalling routes and habituation and visual motor responses to light in a similar way as they do in humans, how fish kairomones and other drugs affect phototaxis across *D. magna* clones and their underlying molecular mechanisms and how UV filters modify the light colour preferences and its ecological implications. Our results indicate that automated imaging enriched methods are promising high-throughput New Approach Methodologies (NAMS) to screen the toxicity of chemicals using ecological and human relevant health responses

2.02.T-02 Data-Driven Characterization of Chemical Impacts on the Genetic Diversity of Wild Populations: A Case Study

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Chemical pollution threatens within-species genetic diversity, and understanding the potential impacts of chemicals is essential to determine the resilience and adaptation potential of different species. However, methods for assessing chemical impacts on the genetics of wild populations are limited, and primarily depend on sequencing large numbers of organisms in a given population/geographic region, which is impractical for all species/populations. Further, finding connections between the genetics of wild populations and chemical exposures is challenging and difficult to do prospectively. We propose that data-driven approaches are essential to help fill the knowledge gap for chemical impacts on the genetics of wild populations. As a proof of concept, we conducted a case study of pesticide impacts on wild boar in Europe to see whether we could identify a link between pesticide concentrations in the environment and differences in wild boar genetic variation. We chose to focus on single polynucleotide polymorphisms (SNPs) as this is the most common type of genetic variation. We collected data on wild boar SNP variation at different sampling sites in Europe (e.g., studies originally conducted to assess wild boar population structure) and maps of modelled soil pesticide concentrations for Europe from the literature. We used a landscape genomics approach to assess the potential relationship between pesticide exposure and wild boar genetic variation. Of the 40,700 SNPs included in the analysis, 2,228 were significantly associated with one or more pesticides, and 702 were intragenic covering 396 unique genes (66 total pesticides were associated with these SNPs/genes). To assess if the SNPs/genes associated with pesticides in the analysis made biological sense, we identified enriched Gene Ontology (GO) terms and found 29 GO terms enriched, including several nervous system-related phenotypes (e.g., neuron to neuron synapse). Because many pesticides are known to have neuronal activity (e.g., malathion, diquat), this provides some support for our predicted associations. To provide further support to the Pesticide SNP/gene associations from our landscape genomics approach, we looked at reported associations from the literature and found external support for pesticides altering gene activity in wild boar. This analysis demonstrates the value of repurposing existing datasets to assess the potential impact of chemicals on genetic variation in populations.

2.02.T-03 Evolutionary Adaptation to Predation Mediates Microplastics Sensitivity in *Daphnia Magna*: The Critical Role of Gut Microbiota

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Microplastic pollution and predation pressure are two key stressors in aquatic ecosystems, but their interactive effects, especially from an evolutionary perspective, are poorly understood. Furthermore, the role of gut microbiota in mediating these combined stressors remains underexplored. This study examines how historical fish predation (pre-fish and high-fish periods) shapes *Daphnia magna*'s tolerance to combined predation and microplastics stressors and explores the role of gut microbiota in modulating these effects. Our findings show that microplastics strongly disrupt the inducible defenses of *Daphnia*, with populations from high-fish environments displayed stronger adaptive responses to predation, which in turn enhanced their tolerance to microplastics when exposed to both stressors. Moreover, gut microbiota were proved crucial in modulating the resilience of *Daphnia* to these combined pressures. High-fish period *Daphnia* exhibit a more resilient gut microbiota, which is characterized by minimal alterations in gut microbial diversity and enhanced functional attributes under stress. This study highlights the importance of evolutionary perspective and the complex interplay of environmental pressures in assessing the ecological risks of microplastics, emphasizing the pivotal role of gut microbiota in mediating these interactions.

2.02.T-04 Determination of Immuno-ecotoxic Effects and Evaluation of their Relevance for Chemical Hazard Assessment

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Climate change, invasive species and habitat fragmentation pose major challenges for the stability of ecosystems. It is assumed that in stressed ecosystems, pathogens in particular exert strong pressure on populations. In addition, the organisms in the environment are exposed to a large number of directly or diffusively released environmental pollutants such as pesticides or drugs. The ecotoxicological hazard and risk assessment of chemicals currently does not include the identification of effects regarding the immunocompetence of organisms. However, studies indicate that chemicals can induce various effects on immune related parameters of organisms. In relation to the increasing pressure from pathogens, immunotoxic effects of chemicals that impair the immunocompetence of organisms could influence host-pathogen-interactions and could pose a significant threat to the stability of ecosystems. Thus, the regulatory authorities claim that methods must be developed to identify immunotoxic modes of action (MoA) of chemicals. The presented project aims at the establishment of standardized methods to identify immunotoxic MoA of chemicals in *Danio rerio* (Zebrafish) and *Tribolium castaneum* (Red flour beetle). Challenge experiments are performed using pathogen-associated-molecular patterns (PAMPs) to induce an activation of the immune system. The test organisms are exposed to selected chemicals with known MoA like drugs with immune suppressive or stimulating properties and are PAMP-challenged after exposure. Molecular fingerprints for immunosuppressive and immunostimulating MoA in the challenged immune system are identified using transcriptomics and proteomics. To add on to the weight of evidence for immunotoxicity, effects on immune related parameters are investigated. In a last step of the project, substances that are suspected to influence the immunocompetence of organisms, for example RNAi pesticides, are tested using the established methods. Furthermore, the effect of these substances on population relevant endpoints are examined in extended ecotoxicological tests and host resistance assays are conducted. The results of the study will contribute to the development of Adverse Outcome Pathways (AOP) for immunotoxicity.

2.02.T-05 Does Thermal Evolution Influence Thermal Performance Curves Under Pollutant Exposure?

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While it is well-known that natural populations can evolve higher thermal tolerance in response to global warming, less attention has been given to how this thermal evolution might influence responses to additional stressors, such as pollution. Although thermal performance curves (TPCs) are valuable and conceptual tools for predicting organismal and population responses to global warming, they have barely been applied to deepen our understanding of population dynamics in a world increasingly stressed by both warming and pollution. In this study, we tested the impact of pollutant exposure (three zinc concentrations) on the thermal performance curve of the water flea *Daphnia magna*, and examined how this was influenced by thermal evolution. To do so, we used clones from two *D. magna* subpopulations (old: 1955 1965 vs recent: 1995 2005) that have different levels of heat tolerance and have been resurrected from the resting bank of a single lake which has experienced an average temperature increase and more frequent heat waves in the recent decades. Overall, both low and high zinc concentrations reduced survival, acute heat tolerance (critical thermal maximum, CTMAX) and body size in both *D. magna* subpopulations. In the absence of zinc, thermal evolution in the recent subpopulation improved performance at the highest temperature (30 °C), with the recent subpopulation showing higher survival and CTMAX compared to the older subpopulation. However, the survival advantage due to thermal evolution was reduced and for heat tolerance, entirely offset by zinc exposure, with the recent subpopulation exhibiting lower survival and CTMAX than the older subpopulation under the combined stress of the highest temperature and zinc exposure. These findings emphasize that the benefits of thermal evolution were weakened for chronic heat tolerance and fully offset for acute heat tolerance under the most stressful combination of warming and zinc exposure. In conclusion, this study provides rare evidence that TPCs, which are widely used to evaluate the impact of global warming on organisms, can be significantly affected by metal exposure. Our results suggest that predictions of population responses to future warming based solely on TPCs may be biased if pollutant exposure is not considered. This underscores the importance of investigating pollutant toxicity across a range of temperatures to generate more accurate forecasts for populations facing a warmer and polluted world.

2.02.P Evolutionary and Mechanistic Insights to Improve Ecotoxicology

2.02.P-Tu163 Evolved Differences in Toxicity: Insights from the *Daphnia pulex*-*Daphnia pulicaria* Complex

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Daphnia are keystone species in aquatic ecosystems commonly used to monitor the impact of chemical pollution. Typically, toxicological assessment utilizes a single clonal isolate of a *Daphnia* species suggesting all *Daphnia* will respond similarly to chemical stressors. However, *Daphnia* have adapted to live in varied habitats around the globe, which shape their unique physiological processes and ecological preferences. In North America the predominant species are *Daphnia pulex* and *Daphnia pulicaria* which are part of a rapidly, radiating clade. They are phenotypically indistinguishable yet evolved to live in different habitats with *D. pulicaria* preferring static lakes, and *D. pulex* preferring ephemeral ponds. These habitat preferences drive important physiological differences of toxicological importance including life span, and reproductive and metabolic rates. A recent study from our lab has shown that only 30% of genome differs between species, while the majority (70%) is homogenized through gene flow. These differences are constrained to large, rearranged regions that persist on each chromosome and harbor genes under selection of these ecological forces. We hypothesize that the fixed differences found within these divergent regions, when probed with a variety of chemical stressors, will reveal the mechanisms controlling the differences in toxicity between the species. Our lab has determined that the triazine herbicide, Atrazine, produces differential acute and chronic responses between these species. Acute exposures revealed *D. pulex* are more sensitive to atrazine when compared to *D. pulicaria*. Chronic atrazine exposures revealed that *D. pulex* experience greater negative impacts on total reproduction in comparison to *D. pulicaria*. Individual responses support our hypothesis; but remain unclear if these responses are linked to the divergent regions. Therefore, this research presents the dissection of the molecular responses in the fixed differences that exist in the divergent genome regions in these species following exposure to atrazine. We hypothesize that these genes and processes that have evolved to prefer different habitats (ephemeral ponds, lakes) such as reproduction, formation reactive oxygen species, antioxidant defense, and lipid peroxidation will respond differently when exposed to Atrazine. This research will provide an example of how evolutionarily driven aspects of the genome can influence toxicological responses.

2.02.P-Tu164 Non-Invasive Automated Approach for Evaluating the Size of Small Aquatic Organisms: *Daphnia magna* Case Study

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In Ecotoxicology, there is an increasing demand for the measurement of sensitive sublethal endpoints, including developmental time, body length and growth rate. The evaluation of body length and subsequent growth rate in small aquatic organisms enables the inference of developmental time, which represents a sublethal endpoint with implications in the life-history of the model organisms. Assessing the body size of aquatic organisms is particularly relevant for *Daphnia* and similar model organisms, fulfilling the requests of chronic toxicity assays, according to OECD guidelines. Currently, the evaluation of the body size of neonates and adults often requires a stereomicroscope observation, either through direct measurement or subsequent measurement of previously captured images. Hence, the objective of this study was to assess the feasibility of using a non-invasive automated computer approach for evaluation of the body length of small aquatic organisms, with *Daphnia magna* at different life stages serving as a case study. The automated computer device (EP3214426B1), besides counting number of organisms, estimates the body size of *D. magna* neonates and adults, from the gravity-driven passage time (intercepting the light path of the sensors), regarding each individual organism that passes the sensors. The accuracy of the computer estimation of *D. magna* body length was obtained by comparing direct measurements (performed under a stereomicroscope, mm) to the device's indirect estimation, which is given by the corresponding passage time (duration of light interference induced by the same organism (μ s)), showing a linear correlation ($y=0.74x$; $r^2>0.92$). Subsequently, a linear high correlation ($y=1.0x$; $r^2>0.92$) was also obtained between the conventional and the new computer-assisted approach for assessing the body length of *D. magna*. Results indicate that the computer assisted evaluation of body length is more straightforward and faster, thus, reducing the high costs and labor intensity associated with following the body length of aquatic small organisms. As a consequence, assessing the body length of an organism can be performed more easily, allowing the measurement over longer timescales, including multiple generations, which are considered to represent natural exposure scenarios more realistically.

2.02.P-Tu165 Sex-specific Vulnerability of Marine, Freshwater and Terrestrial Animals to Pollutants: A Meta-analysis Study

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Chemical pollution is ubiquitous and impacts animals across all realms, marine, freshwater and terrestrial ecosystems. However, chemical pollution may influence males and females differently, which may have consequences for populations, communities and ecosystems. To the best of our knowledge, no systematic reviews or meta-analyses have compared the effect of chemical pollution on females and males. Using the Web of Science Core Collection we conducted a systematic search for relevant publications on this topic in the last decade, from 2010 to 2022. Our search yielded 321 papers after duplicates were removed, and 125 primary research papers after title and abstract screening. Our aim was to explore sex-specific stress responses of exposed males and females. We studied species in different phyla and realms to chemical pollutants such as pesticides, flame retardants, hydrocarbons and microplastics. We further investigated the gene, hormonal and behavioural mechanisms underlying the different sensitivities between the sexes. Preliminary findings show that most of the studies are from the global North and China. Most studies are from the freshwater and marine realm, with most of the organisms tested being a few species of fish, crustaceans, and molluscs. Preliminary results suggest females have higher LC50/LD50, and underlying mechanisms vary between the sexes and species. Moreover, sex ratios can change towards more female or male dominance compared to the control. Our results suggest that pooling the sexes in ecotoxicological studies may confound the results, also because stress responses that alter sex ratios are likely to have long-term consequences for population fitness. We suggest that sex-specific vulnerability to pollutants should be included in ecotoxicological studies whenever possible to improve ecological risk assessments.

2.02.P-Tu166 Decades of E-Waste Recycling Activities Reshaped Local Mosquitofish by Adaptation to AhR Agonists

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Anthropogenic activities, such as electronic waste (e-waste) recycling, have led to severe pollution in aquatic environments with various aryl hydrocarbon receptor (AhR) agonists such as dioxin-like compounds (DLCs). However, the adaptive phenotypes of organisms in these polluted ecosystems remain largely unknown. Here, we identified a mosquitofish population (*Gambusia affinis*) that has resided for over 40 years at an e-waste recycling site and has developed adaptation to an AhR agonist, phenanthrene. This population exhibited a two-fold increase in resistance compared to a mosquitofish population from a reference site, in both F0 adult fish and F1 juvenile fish. The adapted *G. affinis* displayed elevated CYP1A induction both in vitro and in vivo, indicating an alternative adaptive strategy compared to previous studies. This adaptation involved increased biotransformation, rapid toxicant dynamics in vivo, and enhanced swimming behavior, suggesting a link to increased bioenergetic functions. These functions allow for higher energy allocation towards both behavioral activities and phenanthrene detoxification. Our findings underscore the importance of considering multiple levels of organismal effects to predict population and ecological consequences. This distinct DLC adaptation in a freshwater fish species offers valuable insights into forecasting the impacts of radical environmental changes resulting from long-term industrial activities.

2.02.P-Tu167 Sensitivity of Fishes to Aryl Hydrocarbon Receptor Activation by Polycyclic Aromatic Hydrocarbons Follows Phylogenetic Relationships

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Polycyclic aromatic hydrocarbons (PAHs) are structurally diverse organic chemicals known to induce toxicity in fishes by activating the aryl hydrocarbon (AhR). The AhR is a ligand activated transcription factor responsible for regulating a large battery of genes involved in diverse physiological processes. Species sensitivity distributions have been developed for sensitivity to activation of the AhR by the prototypical AhR agonist TCDD, benz[a]anthracene (BAA) and 8-methylbenz[a]anthracene (8-MBAA) that varied by 167-, 141-, and 561-fold, respectively. However, while species can vary dramatically in sensitivity, most research has been performed on a small number of model test species that may not be representative of native species of concern. Currently, no methods for predicting species sensitivity to PAHs have been developed. However, such tools would greatly facilitate risk assessment due to

differences in sensitivity across species, and practical limitations of performing traditional toxicity tests across numerous species. This research aims to 1) continue expanding species sensitivity distributions for sensitivity to AhR activation in additional species and 2) determine whether differences in species sensitivity follow phylogenetic relationships which could then be used to make predictions. This research utilized a standardized in vitro luciferase reporter gene assay to develop species sensitivity distributions based on species-specific half maximal effect concentrations (EC50s) for transactivation of the AhR by TCDD, BAA, and 8-MBAA in 18 species. Phylogenetic signals were then calculated for each compound using Pagel's Lambda (?) and Blomberg's K method for each chemical. Each chemical showed a phylogenetic signal of differing strength. The phylogenetic signal for TCDD was the strongest ($\lambda = 0.99$, $p = 0.004$; $K = 0.69$, $p = 0.048$) while BAA ($\lambda = 0.99$, $p = 0.01$; $K = 0.59$, $p = 0.176$) and 8-MBAA ($\lambda = 0.99$, $p = 0.003$; $K = 0.67$, $p = 0.418$) showed phylogenetic signals that were weaker and did not reach the level of significance using both methods. These results suggest that species that are more closely related phylogenetically can be expected to be similar in sensitivity to AhR activation by each compound which could facilitate predictions for species of unknown sensitivity. However, additional research is needed to generate data from additional species to strengthen this data set and ensure that closely-related species are available for comparison.

2.02.P-Tu168 Exploring the Applicability of the OECD TG 249 Fish Cell Line Acute Toxicity Assay in Environmental Hazard and Risk Assessment of Cosmetic and Personal Care Product Ingredients

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Significant efforts are being made to advance the adoption of animal-free safety assessments. However, acute fish toxicity data are currently a component of environmental hazard and risk assessments and often an explicit requirement in global chemical regulations. To minimize animal use while ensuring the environmental safety of chemicals and products, it is necessary to consider alternative methods for assessing acute fish toxicity that will enable robust and regulatory-compliant animal-free approaches to decision making. One alternative that has shown utility for assessing acute fish toxicity is the OECD TG 249, rainbow trout gill cytotoxicity assay. Although this assay has shown a good correlation with historical standard acute fish toxicity (e.g., OECD TG 203) test data, there have been calls for further data generation with TG 249 to better define the chemical applicability domain and identify potential assay limitations before full implementation within regulatory schemes. To further explore the suitability of this assay and identify best use cases, a project is underway by the not-for-profit science organization ICCS (International Collaboration on Cosmetics Safety) to evaluate the OECD TG 249 against a diverse set of cosmetic and personal care product ingredients. This presentation will serve as an overview of the planned multi-phase approach for the project, provide an update on the status and an opportunity for stakeholders to provide feedback or share relevant findings in either testing or conducting environmental safety assessments using OECD TG 249 data especially with cosmetic and personal care product ingredients. Ideally, this will ensure efficient, appropriate, and strategic use of resources in building a high-quality dataset to support the best use of this assay in NAM (new approach methodology) frameworks for environmental safety assessment and will help expedite broader regulatory acceptance of approaches leveraging this assay. This reach is funded by the International Collaboration on Cosmetics Safety.

2.02.P-Tu169 Comparing Evolutionary AhR2-Activation in Transfected COS-7 Cells Exposed to the Benzotriazole UV-stabiliser UV-P

Andreas N.M. Eriksson¹, Justin Dubiel¹, Cameron Hunter Collins², Hunter Johnson¹, Jonathon Doering² and Steve Wiseman¹, (1)University of Lethbridge, Canada, (2)Louisiana State University, United States Benzotriazole UV-stabilizers (BUVSs) are a class of chemicals added to various consumer products (e.g. lotions, cosmetics, plastics, etc) and utilized in industrial processes. Functionally, BUVSs extend the lifespan of plastics by absorbing light in the UV-A and UV-B spectra. Produced since the 1950s, BUVSs are now be found in every strata across the world as it accumulates ($\log K_{ow}$ typically > 6) in soil, sediment, and tissue. Due to their ability to accumulate, the European Union completed the phaseout of several BUVSs as of 2023, and in the same year, one BUVSs (UV-328) was added to the Stockholm Convention. However, little is known about these chemicals (66 hits on Sciencedirect); although it is known that some BUVSs, including UV-P, can cause toxicity by activating the Aryl Hydrocarbon Receptor 2 of fishes (AhR2). In this study, we investigated activation of the AhR2 of 19 phylogenetically diverse marine and freshwater fish species. COS-7 cells, which lack an endogenous AhR pathway, were transfected with species specific *ahr2* and activation of the receptor was quantified using a luciferase

assay. In parallel, activation of the AhR2 by the prototypical AhR agonist TCDD, was characterized in each species. This study investigates the inverse relationship between the sensitivity of fishes to AhR activation by UV-P and TCDD (based on data from 9 freshwater fishes). Species that were on the sensitive end of the species sensitivity distribution for AhR2 activation by TCDD were on the insensitive end of the species sensitivity distribution for UV-P (Japanese medaka, *Oryzias latipes*; TCDD: 0.3 nM compared to 30000 nM for UV-P) and vice versa (white sucker, *Catostomus commersonii*: TCDD 1 nM compared to 300 nM for UV-P). Additionally, we will compare the sensitivity of each species with their evolutionary relatedness to draw conclusions on how evolution predicts taxa specific sensitivity to AhR2 activation. This research has implications for risk assessment as species thought to be tolerant to UV-P due to their tolerance to TCDD might be at greater risk of AhR2 mediated toxicity than previously thought.

2.03 Linking Lab and Field Evidence on Pesticide Effects on Biodiversity and Ecosystem Functions

2.03.T-01 Revealing the Cascade of Pesticide Effects from Genes to Communities

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Agricultural pesticides, even at concentrations below regulatory thresholds, are significant drivers of biodiversity loss, altering ecosystems from genetic to community levels. Freshwater ecosystems are particularly vulnerable due to proximity to agricultural runoff. This study investigates the cascading effects of pesticide exposure on *Gammarus pulex*, a dominant freshwater crustacean, and its associated macroinvertebrate communities across streams in central Germany. Pesticide exposure was quantified, and genetic diversity in *Gammarus pulex* populations was assessed using molecular techniques. Laboratory experiments measured fecundity and tolerance to neonicotinoid pesticides, while community-level impacts were evaluated through field surveys of macroinvertebrate diversity and composition. Results reveal that populations exposed to higher pesticide contamination exhibited reduced genetic diversity and altered allele frequencies, indicating strong selective pressure. These genetic adaptations were linked to increased pesticide tolerance but incurred fitness trade-offs, such as reduced fecundity in pesticide-free environments. At the community level, pesticide exposure reduced the abundance of sensitive species, such as Ephemeroptera, Plecoptera, and Trichoptera, and reshaped macroinvertebrate composition. This loss of competition allowed pesticide-tolerant *Gammarus pulex* populations to dominate, despite their reduced fitness. Streams closer to uncontaminated refuges retained higher genetic diversity and lower frequencies of pesticide-tolerant alleles, demonstrating the protective role of refuge areas. This research underscores the need to integrate genetic and ecological perspectives into chemical risk assessments, which often overlook the sublethal and long-term impacts of low-level pesticide exposure. By linking genetic changes to ecosystem-level consequences, this study highlights the broader implications of chemical contamination on biodiversity and ecosystem functionality, urging a more comprehensive approach to pesticide regulation.

2.03.T-02 A Streamlined Approach to Pesticide Risk Assessment that Aligns Prediction and Reality

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The environmental risk assessment is initially carried out by using standardised acute or chronic test systems and deriving endpoints to which assessment factors were added in order to protect communities in the field. However, it became clear that effects at ecosystem level are also determined by a large number of other ecological factors and processes. These are better addressed in higher tier effect assessment such as in model-ecosystems. With the expanded knowledge of the effect-determining factors and mechanisms, it became clear that a practicable and predictive risk assessment taking into account the entire complexity of the cause-effect relationships not feasible for prospective regulatory contexts based on predictive approaches is not possible. The EU Partnership for the Assessment of Risks from Chemicals (PARC), activity 6.4.4 aims to advance chemical risk assessment to protect human health and the environment. Here we suggest a new approach for a protective ERA with reduced complexity and resources required while keeping it as information-rich as necessary under Regulation (EU) 546/2011. We compare a common Effect-Cascade Prediction approach and the novel Effect-Adaptation Prediction. Effect-Cascade Prediction: The common approach in ecological prediction to identify effect assessment at ecosystem level. Here, the effects of various environmental factors and stressors on an individual are predicted. This can then be extrapolated to populations or communities Effect-Adaptation Prediction: This approach enables a prospective effect assessment at ecosystem level. The pesticide toxicity information is revealed with standard laboratory experiments. The prediction of the effect in the field is based on pesticide-specific indicators, generally based on ecological traits. The link between results from laboratory test systems and the effects at ecosystem level is initially established through exposure and effect monitoring of some widely used pesticides. I will present these different approaches and discuss their respective

advantages and disadvantages. The author thank the EU PARC regulatory core group for intensive discussion. This work was supported by the German Helmholtz Research (POF IV, Topic 9 Healthy Planet) and the European Partnership for the Assessment of Risks from Chemicals (PARC), supported by European Union s Horizon Europe research and innovation programme under grant agreement no. 101057014.

2.03.T-03 Benthic Diatoms as Indicators of Pesticide Pollution in Swiss Watercourses

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Protecting aquatic ecosystems from pesticide pollution and other micropollutants is a critical regulatory task. Benthic diatoms, commonly used to indicate nutrient pollution, exhibit variable species sensitivities to pesticides. Therefore, we assess if they could also serve as effective bioindicators for pesticide pollution. To identify potential benthic diatom taxa that are sensitive or tolerant to pesticides, we conducted a laboratory study using flow-through chambers for periphyton colonisation in a closed and controlled system. We continuously exposed the periphyton to 44 treatments, consisting of combinations of environmentally relevant pesticide levels (36 levels of Toxic Unitmix ranging from 0 to 4.6) and three environmental factors (i.e., light intensity, temperature, and nutrient levels) over a 23-day colonisation period. Following colonisation and exposure, we examined the diatom community composition and structure using both morphological and molecular techniques to address discrepancies between the two approaches. The similarities in diatom community composition assessed using ordination techniques revealed no significant correlation with pesticide stress, while light intensity and temperature showed a significant impact. A multivariate beta regression model was used to quantify the effect of pesticide stress and the other environmental factors on the relative abundance of diatom species, highlighting both similar and distinct patterns of tolerance and sensitivity for a set of species observed across the two identification techniques (morphological and molecular). Community-level periphyton functions and structure (i.e., effective photosynthetic activity, chlorophyll-a content, ash-free dry weight, and bacterial abundance) were assessed using a linear model where pesticide treatments showed no significant impacts. This could be due to functional redundancies within the diatom communities that may prevent significant impacts on functions at environmental pesticide concentrations. These findings suggest that certain diatom species could be promising bioindicators for pesticide pollution. Further research at the community level and with single species are required to validate this hypothesis. Ultimately, this project could aid in developing diatom-based indicators, improving pesticide impact assessment and ecosystem management, while addressing discrepancies between morphological and metabarcoding approaches for diatom identification.

2.03.T-04 Behavioural Effects of Pesticide Mixtures and Caffeine on *Hyaella azteca*

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Residues of agricultural and urban contaminants occur frequently in freshwater bodies. They cause negative impacts on the freshwater organisms by altering their reproduction, food consumption, behaviour and, consequently, their ecological interactions. Mixtures of substances can lead to even more severe damage. At the same time, the individual effects are difficult to assess. In this study, the epibenthic crustacean specie *Hyaella azteca* was used to observe changes in activity behaviour using the Multispecies Freshwater Biomonitor. The response of *H. azteca* to the pesticides tebuconazole, nicosulfuron, chlorpyrifos and clothianidin, as well as their combinations was tested. Furthermore, pesticide interaction with the urban contaminant caffeine were of particular interest because of its ubiquity in the aquatic environment. Our results indicate that all pesticides, as well as their mixtures, have a statistically significant effect on the activity of *H. azteca*. The observed variation in response to contaminants results from different mode of action, which range from escape behaviour to a complete reduction in swimming rates. Contrary to the effect observed on humans, caffeine lowered the activity level of *H. azteca* up to 17% in single substance treatments. However, further studies are needed to truly understand the mode of action of the tested substances and their impact on the activity behaviour of crustacean. This study was part of the German Academic Exchange Service scholarship no. 57725695.

2.03.P-We046 Behavioural Response of a Native and Invasive Amphipod to Sublethal Pesticide Exposure

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Aquatic ecosystems are subject to a multitude of environmental pressures simultaneously, including chemical toxicants and biological stressors. In freshwater habitats, chemical contamination and invasive species are often considered prominent drivers of biodiversity decline. However, the ecological consequences can potentially be more pronounced when these stressors interact. Therefore, this study aimed to assess the combined effect of pesticide inputs and invasive species on ecosystem resilience and functioning. Behavioural endpoints were used to assess the impact of pesticide exposure on two amphipod species, the native *Gammarus duebeni celticus* and the invasive *Gammarus pulex*. Each species was subject to sublethal concentrations (1, 5 & 10 µg/L) of the phenoxyacetic acid herbicide, 2-methyl-4-chlorophenoxyacetic acid (MCPA) for 96h to investigate the acute effects of exposure. Despite their widespread and extensive use, phenoxyacetic acid herbicides remain understudied with respect to their toxicological effects. Species differences were assessed by employing the comparative functional response (CFR) metric, which quantifies resource use as function of prey density. FR trials were conducted using live chironomid prey (*Chironomus* sp.). Given the status of *G. pulex* as an established invader in this instance, a greater FR in comparison to *G. duebeni* was expected. The CFR is an effective means by which to determine species response to contaminant exposure and ecosystem impacts of invaders. Incorporating indirect effects, such as species interactions, in the assessment of chemical contaminants provides a more holistic understanding of ecosystem level impacts.

2.03.P-We052 Effects of the Fungicide Tebuconazole with Endocrine Acting Potential on a Macroinvertebrate Community in Stream-Lake Mesocosms

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Understanding the consequences of increased pesticide use in agriculture is crucial to protecting terrestrial and aquatic ecosystems from harm. Tebuconazole (TBZ) is a triazole fungicide that inhibits the biosynthesis of ergosterol in fungal cell membranes and thus fungal growth. In addition, TBZ blocks the biosynthesis of cholesterol as an important precursor of sexual steroid hormones in animals, and can therefore act as endocrine disruptor. As part of a comprehensive indoor mesocosm study, aimed at revealing effects of TBZ on fungal biodiversity and functioning, we also investigated the effects of TBZ on the macroinvertebrate community and insect emergence in stream-lake mesocosms over a 7-month period. Three and one month prior to TBZ application, macroinvertebrates from an unpolluted stream were transferred into the freshwater mesocosms for acclimatization, using attraction devices consisting of organic straw. The mesocosms were dosed once with six concentrations of TBZ using the formulation Folicur® (5, 50, 100, 500, 1000, 5000 µg/L TBZ). Two mesocosms served as controls. The macroinvertebrate community showed community response (principle response curve analysis) at concentrations higher than 100 µg/L TBZ. Gammarids and snails were the most sensitive taxonomic groups in the systems, while species of chironomid sub families differed strongly in their sensitivity. For the endpoint emergence, number of male chironomids decreased after TBZ application, whereas the number of females even increased in the 500 and 1000 µg/L TBZ treatments. This result may be attributable to the endocrine acting potential of TBZ. Transferring field macroinvertebrate communities into mesocosms seems to be a suitable method for investigating TBZ effects on benthic organisms. We could identify direct and indirect effects of TBZ on macroinvertebrates, underlining the added value of mesocosm studies for risk assessment of pesticides in aquatic environments.

2.03.P-We061 Evaluation of Triazole Fungicide Exposure to the Development of *Tenebrio molitor*

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The use of pesticides, in particular triazole fungicides, contributes significantly to biodiversity loss in agroecosystems. These fungicides affect fungal cell wall formation by inhibiting ergosterol synthesis, and in arthropods they can disrupt the production of ecdysteroids, key hormones in moulting and reproduction. Since invertebrates are essential for ecosystem balance, this study evaluated the effects of triazoles on the mealworm (*Tenebrio molitor*) by simulating exposure scenarios through the ingestion of cereal seeds treated with prothioconazole and tebuconazole. Physiological variables (size, weight and development) and changes in gene expression linked to ecdysteroidogenesis were measured. Three exposure treatments were carried out: seeds treated with the labelled dose of Raxil® Plus, 50:50 and 25:75 mixtures of treated and untreated seeds, and a control with untreated seeds. Sixty larvae per treatment were exposed for 10 days, ingesting only the allocated seeds. Subsequently, they were offered oats, wheat bran and vegetables,

and their development was monitored until the adult stage. Weight, mortality, malformations and development time were recorded. For molecular analysis, total RNA was extracted from larvae, pupae and adults and converted into cDNA for qRT-PCR. Gene expression was assessed using specific primers and relative differences from the control were calculated. Mealworm showed rejection of treated seeds, especially in the intermediate treatment. A decrease in weight was also observed in the higher treatments. No significant differences in development time were observed. Gene analysis revealed a lower expression of the enzymes Spo, Sad, Shd and the transcription factor HR3 in the highest treatment. In the intermediate treatment, a reduction of Spo and HR3 was detected, as well as an overexpression of the regulator E75. These changes suggest that fungicides interact with key elements of the ecdysteroidogenesis pathway, which could compromise essential functions in exposed arthropods. In conclusion, exposure to triazole fungicides may affect both physiological parameters and genetic regulation of hormonal processes in *Tenebrio molitor*, highlighting the need to assess their impact on biodiversity and agroecosystem functioning.

2.03.P Linking Lab and Field Evidence on Pesticide Effects on Biodiversity and Ecosystem Functions

2.03.P-We042 Effects of the Antimicrobial Triclosan on Community Structure and Ecosystem Functioning in Sub-tropical Freshwater Ecosystems

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Triclosan, a synthetic broad-spectrum antimicrobial agent, is commonly used in personal care products, household goods, and industrial items. Its extensive use and subsequent release have led to frequent detections in aquatic ecosystems worldwide, potentially harming ecosystem health. Here, an outdoor mesocosm experiment was conducted to assess the effects of triclosan on community structure (microbes, phytoplankton, zooplankton, and macroinvertebrates) and ecosystem functioning in freshwater ecosystems under sub-tropical conditions. Triclosan was applied daily in pulses at nominal concentrations of 0.05, 0.5, 5, and 50 µg/L over a three-week treatment period, followed by a five-week recovery period. Triclosan significantly affected the structure of microbial (NOEC of 0.5 µg/L), phytoplankton (NOEC of 5 µg/L), and zooplankton (NOEC of 5 µg/L) communities. Within the microbial community, the phyla Cyanobacteria and Bacteroidetes consistently showed significant responses to triclosan exposure, with taxa in the Cyanobiaceae family being the most adversely affected. Among phytoplankton taxa, *Cylindrospermopsis* sp. had the lowest consistent NOEC (< 0.05 µg/L), while the lowest NOEC (0.5 µg/L) among zooplankton taxa was observed for nauplii and *Mesocyclops leuckarti*. Piecewise structural equation modelling revealed that triclosan exposure resulted in significant direct and indirect effects across multiple trophic levels via cascading interactions, ultimately influencing ecosystem functioning related to carbon and nitrogen cycling. Hence, our study highlights the threat posed by environmentally relevant concentrations of triclosan to both community structure and ecosystem functioning in sub-tropical freshwater ecosystems.

2.03.P-We043 Towards the Integration of Periphyton in Pesticide Biomonitoring to Assess Ecological Impacts on Agricultural Streams

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In agricultural streams aquatic biofilms (hereafter periphyton) are exposed to several stressors including a wide array of pesticide residues. This study investigated the temporal variations and site differences in pesticide concentrations accumulated in periphyton over the course of three months. In addition, we assessed if changes in the structure of the algal community and the fatty acid content as proxy of periphyton nutritional value were linked to pesticide exposure in periphyton. Field experiments were conducted in two agricultural streams and one reference stream in Sweden during summer 2022. Artificial substrata (tiles) were deployed in each stream to facilitate periphyton colonization for three months. Periphyton samples were collected once per month. Surface water was collected with weekly time -

integrated sampling. Analysis included residue of 109 pesticides, in surface water and in periphyton, 37 fatty acids, ash-free dry mass, and 11 photosynthetic pigments in periphyton. In periphyton, up to 31 pesticides were detected, with distinct chemical profiles observed in the two agricultural streams. The pesticide composition in periphyton differed from that in the water. Water profiles showed greater variability over time and were dominated by water-soluble substances. In contrast, periphyton accumulated more hydrophobic compounds, some of them banned for years, suggesting longer-term, chronic exposure. Pigment analyses revealed high algal biomass, predominantly composed of diatoms, but lower diversity in the agricultural streams compared to the reference stream. Diatom biomass was strongly correlated with the polyunsaturated fatty acids EPA and DHA concentrations, markers of high nutritional value produced mainly by diatoms. Linear regression revealed a general, though not significant, negative relationship between pesticide concentrations in periphyton and diatom biomass or EPA levels. We hypothesize that at low pesticide concentrations, other stressors, such as high nutrient load, are more prominent. However, beyond a certain threshold, pesticide exposure may become a primary stressor, reducing nutritional quality of the periphyton and altering algal community. These findings highlight that periphyton in agricultural streams accumulate a variety of pesticides, exhibit high biomass and display altered algal community structure and fatty acid profiles.

2.03.P-We044 Investigating the Role of Pesticides and Pharmaceuticals in Riparian Ecosystems: Insights from the RIPARIANET Project

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Riparian zones are natural ecotones that contribute greatly to biodiversity and ecosystem services. As Europe commits to protecting 30% of its land and sea, riparian networks "blue-green arteries" connecting ecosystems across elevation gradients offer critical opportunities for targeted conservation. The RIPARIANET project focuses on six river basins across Europe, covering the boreal (Sweden), continental (Germany), alpine (Trento, Italy), Mediterranean (Rome, Italy), and Atlantic (Northern Spain, Northern Portugal) climatic regions to optimize conservation strategies for these ecosystems. This study investigates how pesticides and pharmaceuticals entering riparian ecosystems through water affect aquatic and terrestrial food webs. By examining contaminant transport and accumulation across water, biofilms, emerging insects, riparian spiders, and bats, we aim to assess ecosystem-level impacts and distribution of contaminants across trophic levels. Our approach combines GIS-based modeling with comprehensive field data collected in 2024. We analyzed a wide range of contaminants in field-collected samples using UHPLC-ESI-MS/MS. Our findings will shed light on how pesticides and pharmaceuticals are transported into aquatic and terrestrial food webs and their potential impacts on biodiversity and ecosystem resilience. As environmental stressors such as chemical pollution interact with broader challenges like climate change, understanding these dynamics is crucial for developing conservation strategies that protect riparian ecosystems under future conditions. Biodiversa+ RIPARIANET project

2.03.P-We045 Effects of Flood-Mediated Pollutant Transfer on Total Polyphenol Content in Riparian Plants

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Flooding can transfer pollutants from aquatic to terrestrial ecosystems, acting as a contamination pathway for riparian plants. Due to land-use changes and more frequent and intense rainfall events caused by climate change, both flooding frequency and duration are expected to rise over the next decades. This increase will intensify the floods ability to act as an exposure pathway to the riparian vegetation, where agricultural pesticides are transported back to land in riparian zones. This strongly affects the vegetation stress-mediated response, where polyphenolic compounds are involved in defense mechanisms against biotic and abiotic stressors. Research on contaminants entering riparian lands remains limited, especially regarding their impact on plant resilience. This study investigated the effect of flood-mediated pesticide transfer under different flooding frequencies (after 1 and 4 floods) and durations (0, 3, 7 and 14 days) on

the Total Polyphenolic Content (TPC) as a stress biomarker of the stinging nettle (*Urtica dioica* L.) and a common grass species (*Elymus* spp) at the Riparian Stream Mesocosm site in Landau, Germany. We hypothesized that prolonged and frequent flooding will increase pesticide concentration in common grass and stinging nettle, showing a positive correlation with TPC levels as a response to the stress. The concentrations of pesticides and their metabolites were quantified using HPLC-ESI-MS/MS, while TPC was measured spectrophotometrically via the Folin-Ciocalteu reagent method. Ten pesticides were frequently detected in both riparian plants, showing a positive trend between flooding frequency and pesticide concentration. TPC concentrations in grass differed significantly from nettle after flooding, with grass exhibiting higher TPC levels. While no correlations were found between overall pesticide exposure and TPC, specific pesticide compounds showed distinct correlations with TPC, indicating compound-specific responses. These findings highlight the complexity of the impact of flooding and pesticide exposure on riparian plants resilience. Our study suggests that individual pesticide compounds may influence plant biochemical stress responses individually.

2.03.P-We046 Behavioural Response of a Native and Invasive Amphipod to Sublethal Pesticide Exposure

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Aquatic ecosystems are subject to a multitude of environmental pressures simultaneously, including chemical toxicants and biological stressors. In freshwater habitats, chemical contamination and invasive species are often considered prominent drivers of biodiversity decline. However, the ecological consequences can potentially be more pronounced when these stressors interact. Therefore, this study aimed to assess the combined effect of pesticide inputs and invasive species on ecosystem resilience and functioning. Behavioural endpoints were used to assess the impact of pesticide exposure on two amphipod species, the native *Gammarus duebeni celticus* and the invasive *Gammarus pulex*. Each species was subject to sublethal concentrations (1, 5 & 10 µg/L) of the phenoxyacetic acid herbicide, 2-methyl-4-chlorophenoxyacetic acid (MCPA) for 96h to investigate the acute effects of exposure. Despite their widespread and extensive use, phenoxyacetic acid herbicides remain understudied with respect to their toxicological effects. Species differences were assessed by employing the comparative functional response (CFR) metric, which quantifies resource use as function of prey density. FR trials were conducted using live chironomid prey (*Chironomus* sp.). Given the status of *G. pulex* as an established invader in this instance, a greater FR in comparison to *G. duebeni* was expected. The CFR is an effective means by which to determine species response to contaminant exposure and ecosystem impacts of invaders. Incorporating indirect effects, such as species interactions, in the assessment of chemical contaminants provides a more holistic understanding of ecosystem level impacts.

2.03.P-We047 Effects of Triticonazole on a Simple Freshwater Ecosystem under Elevated Temperature

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This study investigates the ecological effects of triticonazole on freshwater ecosystem under elevated temperature condition in a microcosm experiment being conducted from September 30 to November 30, 2024. The experimental design comprises 17 microcosms, including 5 controls and 12 treatment microcosms with three triticonazole concentrations (Low, 10 µg/L; Medium, 100 µg/L; High, 1000 µg/L). Each treatment concentration is replicated four times, while the control treatment has 5 replicates. All microcosms are maintained at a consistent elevated temperature of 28°C to simulate tropical conditions. Key physico-chemical parameters including temperature, pH, electrical conductivity, dissolved oxygen, and nutrients (nitrate, phosphate, ammonium, particulate organic nitrogen, particulate organic phosphorus, and particulate inorganic phosphorus) are being regularly monitored. Biological assessments include the growth of the macrophyte *Myriophyllum spicatum*, the zooplankton community, chlorophyll-a (as a proxy for algal biomass), microbes, and the dynamics of the macroinvertebrates *Gammarus pulex* and *Physella* spp. Feeding rates of macroinvertebrates and microbial leaf litter decomposition rates are being measured to assess ecosystem functionality under triticonazole stress. Water and sediment samples are being collected regularly to evaluate the persistence and distribution of triticonazole within the microcosms. Multivariate analyses using Canoco software will be employed to investigate relationships among physicochemical and biological parameters under triticonazole exposure. This approach will identify patterns and interactions within the data, offering deeper insights into ecosystem responses to triticonazole. This comprehensive analysis aims to provide critical insights into the effects of triticonazole exposure under tropical conditions.

2.03.P-We048 The effects of commercial 2,4-D herbicide on larval fathead minnows: Natural lake water vs. laboratory system water

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Herbicides with the active ingredient 2,4-Dichlorophenoxyacetic acid (2,4-D) are commonly applied worldwide to control non-native aquatic plants that negatively impact native biodiversity and ecosystem services. Traditional laboratory ecotoxicity studies associate exposure to ecologically relevant concentrations of 2,4-D with decreased survival and sublethal impacts on non-target organisms, including multiple freshwater fish species. Given the diverse water parameters of freshwater aquatic ecosystems globally, it is important to evaluate if controlled laboratory experiments reflect toxicity outcomes under real-world scenarios. To do so, concurrent in situ and laboratory exposure assays were conducted. In-situ systems were established in two Wisconsin lakes (Big Lake and Dutch Hollow Lake) one day prior to whole-lake treatment with DMA®4IVM (46.3% 2,4-D) and four months post treatment for control. Larval fathead minnows (*Pimephales promelas*; n = 200 per lake) developed for 21 days, then survival, length, and weight were recorded. Laboratory exposure systems were conducted in parallel using water sourced from the respective lake before and after herbicide treatment. For both in situ and laboratory exposures, a statistically significant decrease in survivorship but no differences in morphological parameters of surviving larvae were observed for 2,4-D treatment groups compared to corresponding controls. No statistically significant differences in survivorship between laboratory controls and in-situ controls were detected for either lake; however, a statistically significant decrease in survivorship was observed for Dutch Hollow in-situ treatment compared to laboratory treatment. Results confirm previous findings of decreased larval fish survivorship upon exposure to 2,4-D in controlled laboratory system water environments, demonstrating the ecological relevance of laboratory studies for elucidating toxicity impacts to non-target organisms in natural lake water. While laboratory studies provide useful information for herbicide risk assessment, it is important for management agencies to take location-specific water parameters and environmental factors into account to more accurately predict and minimize health impacts in non-target species.

2.03.P-We049 Weight-of-Evidence Approaches for Interpreting Multiple Mesocosm Studies: A Case Study with *Lambda-cyhalothrin*

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Experimental ecosystem (mesocosm or microcosm) studies are a valuable refinement tool in aquatic risk assessment for PPPs, accommodating environmental fate and behaviour of the active substance while addressing ecological processes such as community level effects and recovery. These processes occur in real world edge of field surface waters, and mesocosms therefore act as the surrogate reference tier, supporting prediction of risks to aquatic flora and fauna arising from real-world uses of PPPs. While recovery is recognised as an important factor in assessing risk at population and community level, as provided for in the specific protection goals, at the present time regulatory acceptance of recovery arguments is uncertain. Lambda-cyhalothrin (LCYH) is a pyrethroid insecticide that has been used in Europe for over thirty years, with proven efficacy over a wide range of crop-pest combinations. LCYHs neurotoxic mode of action is targeted to crop pests, but also affects non-target arthropods and crustaceans and in Europe at least, the aquatic risk assessment for LCYH invariably requires higher tier refinement for these groups. LCYH has one of the most comprehensive mesocosm datasets of any AI registered in the EU, with the most recent study conducted in 2018 and can be considered state of the art by reference to current best practice. This database provides an exceptional opportunity to explore weight of evidence approaches where multiple mesocosm studies are available and should provide greater certainty in predicting effects in edge-of-field surface waters.

2.03.P-We050 Short-Term Toxicity of Agricultural Pesticides Imidacloprid and Tebuconazole in Three Freshwater Invertebrates: A Comparative Study

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The widespread use of imidacloprid and tebuconazole has led to their frequent detection in surface waters, where their persistence poses risks to non-target organisms, highlighting the need to assess their ecotoxicological effects on aquatic invertebrates. In this context, short-term toxicity tests were conducted on three species of aquatic crustaceans: *Daphnia similis*, *Ceriodaphnia silvestrii*, and *Hyaella meinerti*.

Toxicity tests were conducted for 48 hours at 24 ± 2 °C with a 12L:12D photoperiod for *C. silvestrii*, following ABNT 13373 guidelines, and at 20 ± 2 °C with a 12L:16D photoperiod for *D. similis*, in accordance with ABNT 12713. For *H. meinerti*, tests were performed over 96 hours at 24 ± 2 °C with a 12L:12D photoperiod, following ABNT 15470 guidelines, with feeding provided at the start and after 48 hours. For each species, five concentrations of imidacloprid and tebuconazole were tested, with stock solution concentrations confirmed by liquid chromatography coupled with tandem mass spectrometry, and at least three independent trials were conducted for each species, ensuring a coefficient of variation of less than 20%. For the pesticide tebuconazole, *D. similis* exhibited an LC50-48h of 9.4 mg L⁻¹, with a confidence interval of 8.0 to 10.8 mg L⁻¹. *C. silvestrii* showed an LC50-48h of 3.9 mg L⁻¹, with a confidence interval of 2.8 to 4.9 mg L⁻¹. For *H. meinerti*, the LC50-96h was 6.2 mg L⁻¹, with a confidence interval of 4.1 to 6.1 mg L⁻¹. Regarding the pesticide imidacloprid, *D. similis* exhibited an LC50-48h of 4.1 mg L⁻¹, with a confidence interval of 2.5 to 5.6 mg L⁻¹. *C. silvestrii* showed an LC50-48h of 2.5 mg L⁻¹, with a confidence interval of 2.1 to 2.8 mg L⁻¹. For *H. meinerti*, the LC50-96h was 38.6 µg L⁻¹, with a confidence interval of 21.8 to 44.7 µg L⁻¹. When comparing the two pesticides, imidacloprid was found to be more toxic than tebuconazole to *H. meinerti* and *C. silvestrii*, indicating a greater risk for these species. The varying sensitivity levels among the species highlight the need to use multiple test organisms to better assess the ecotoxicological effects of pesticides. These findings contribute to the field of ecotoxicology by providing new data on the acute toxicity of imidacloprid and tebuconazole to aquatic invertebrates. Such data could contribute to environmental policy, particularly regarding pesticide regulation and the protection of aquatic ecosystems. This research was supported by the São Paulo Research Foundation - FAPESP (grant no. 2022/12104-4). David Silva Alexandre has a doctoral scholarship supported by FAPESP (grant no. 2024/00791-2). Thandy Junio da Silva Pinto and Diego Gomes Ferreira have a postdoctoral scholarship supported by FAPESP (grant no. 2022/14293-9 and 2024/08039-8).

2.03.P-We051 Effect of the Fungicide Tebuconazole With Herbicidal Mode of Action on Plankton and Periphyton Communities in Freshwater Mesocosms

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The fungicide tebuconazole (TBZ) is used against fungi that are pathogenic for agricultural crop. TBZ is also used as growth regulator in agriculture due to its herbicidal mode of action by inhibiting the sterol 14 α -demethylase, which is involved in the synthesis of phytosterols. Therefore, TBZ can potentially have adverse effects on aquatic autotrophs after run-off into freshwater ecosystems. However, there is very limited knowledge on the effects of TBZ on aquatic non-target organisms, such as phytoplankton and periphyton species. In a comprehensive aquatic mesocosm experiment, studying the effects of TBZ on aquatic fungi, we investigated likewise the effects of the fungicide on plankton and periphyton communities. Freshwater mesocosms simulating stream-lakes were inoculated several times with field-sampled plankton and periphyton communities during a 5-month establishing period prior to the fungicide application. The mesocosms were also stocked with macrophytes, aquatic fungi and macroinvertebrates in the establishing period. Using the formulation Folicur®, six concentrations of TBZ (5, 50, 100, 500, 1000, 5000 µg/L) were applied once in the mesocosms and plankton and periphyton communities were sampled regularly after the application for up to 8 months. Principal response curve analyses revealed that the communities of phytoplankton, zooplankton and periphyton responded to the TBZ application gradient. We did not observe an immediate dose-response relationship on community composition. Instead, communities in the mesocosms with TBZ concentrations >100 µg/L tended to show increasingly different species compositions over time, suggesting indirect effects playing a role in driving community responses. Some species were more sensitive to the TBZ treatment than others from the same taxonomic group, indicating inter-specific differences in the response to pollutants and demonstrating the added value of mesocosm studies for risk assessment of chemicals in aquatic environments.

2.03.P-We052 Effects of the Fungicide Tebuconazole with Endocrine Acting Potential on a Macroinvertebrate Community in Stream-Lake Mesocosms

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Understanding the consequences of increased pesticide use in agriculture is crucial to protecting terrestrial and aquatic ecosystems from harm. Tebuconazole (TBZ) is a triazole fungicide that inhibits the biosynthesis of ergosterol in fungal cell membranes and thus fungal growth. In addition, TBZ blocks the

biosynthesis of cholesterol as an important precursor of sexual steroid hormones in animals, and can therefore act as endocrine disruptor. As part of a comprehensive indoor mesocosm study, aimed at revealing effects of TBZ on fungal biodiversity and functioning, we also investigated the effects of TBZ on the macroinvertebrate community and insect emergence in stream-lake mesocosms over a 7-month period. Three and one month prior to TBZ application, macroinvertebrates from an unpolluted stream were transferred into the freshwater mesocosms for acclimatization, using attraction devices consisting of organic straw. The mesocosms were dosed once with six concentrations of TBZ using the formulation Folicur® (5, 50, 100, 500, 1000, 5000 µg/L TBZ). Two mesocosms served as controls. The macroinvertebrate community showed community response (principle response curve analysis) at concentrations higher than 100 µg/L TBZ. Gammarids and snails were the most sensitive taxonomic groups in the systems, while species of chironomid sub families differed strongly in their sensitivity. For the endpoint emergence, number of male chironomids decreased after TBZ application, whereas the number of females even increased in the 500 and 1000 µg/L TBZ treatments. This result may be attributable to the endocrine acting potential of TBZ. Transferring field macroinvertebrate communities into mesocosms seems to be a suitable method for investigating TBZ effects on benthic organisms. We could identify direct and indirect effects of TBZ on macroinvertebrates, underlining the added value of mesocosm studies for risk assessment of pesticides in aquatic environments.

2.03.P-We053 Functional and Behavioral Responses of *Physa acuta* Snails Exposed to Fungicide Tebuconazole

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After being applied to crops, pesticides can be carried to aquatic environments, affecting indigenous organisms. This is the case of tebuconazole, a triazole fungicide that, despite being used to control fungi, may endanger non-target species. In this way, this study investigates how short and prolonged exposure to environmentally relevant concentrations of tebuconazole impairs functional and behavioral responses in *Physa acuta* snails. Chronic and subchronic assays were carried out with five concentrations of tebuconazole (25, 50, 100, 200, and 400 µg/L). The subchronic assays (10 days) were carried out with newborns (1-24 hours life) to assess the effects on the initial growth. The chronic assay (21 days) was initiated with sexually mature individuals (30-32 days live) and investigated responses in reproduction (fertility and fecundity). The displacement behavior (distance traveled and maximum velocity achieved) was assessed at the end of both experiments. All experiments were carried out at 25 °C and 12h:12h (light: dark) light regime and data were evaluated by Generalized Linear Models. In none of the assays, tebuconazole exposure impaired snails survival; thus, sublethal responses were assessed. In the subchronic assay, the fungicide reduced the initial growth of *P. acuta* neonates at all tested concentrations (NOEC < 25 µg/L, $p < 0.05$) relative to control (26 to 90% reduction). Regarding reproductive output from chronic tests, exposure to all tebuconazole concentrations reduced the number of ovigerous masses (46 to 62%) and total of eggs produced (40 to 70%) compared to control individuals (NOEC < 25 µg/L, $p < 0.05$). Also, the egg hatchability was reduced in these same concentrations. To the behavioral responses, newborns were most susceptible to tebuconazole exposure. While the distance traveled and maximum speed was impaired in individuals from all tested concentrations (NOEC < 25 µg/L) in subchronic exposure, only adults from the three highest concentrations (NOEC = 50 µg/L) had these parameters reduced ($p < 0.05$). Despite the action mechanisms of tebuconazole being described to fungi, results demonstrate that exposure to this fungicide at concentrations already reported in aquatic environments impacts functional and behavior traits of neonates and adults of *P. acuta* with several consequences to the maintenance of species in the environment and their ecological interactions at population and community levels. T.J.S.P. would like to thank the São Paulo Research Foundation Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the post doctorate grant (Process number: 2022/14293-9). Financial support was also provided by FAPESP (thematic project 2022/12104-4).

2.03.P-We054 Use of a Native Amazonian Ostracod in the Evaluation of the Sublethal Effects of Tebuconazole and Imidacloprid

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The excessive use of pesticides currently poses a risk to the health of aquatic biota, since different pesticides inevitably end up reaching aquatic ecosystems. Therefore, this study aims to evaluate the chronic effects of environmentally relevant concentrations of imidacloprid and tebuconazole on a native species of ostracod, *Strandesia rondoniensis*. *S. rondoniensis* cultures were maintained following recommendations adapted from the ABNT protocol for tropical cladocerans, No. 13373. Feeding

consisted of 20 mL of Tetramin® fish food (5 g solution in 1 liter of distilled water) and 20 mL of *Raphidocelis subcapitata* suspension ($\approx 10^5$ cells L⁻¹). The experiments were carried out at $25 \pm 1^\circ\text{C}$ with a 12L:12D photoperiod. Each pesticide had 8 replicates per concentration, with one juvenile individual per replicate, and a duration of 20 days. The concentration ranges used in the tests were: 0 / 22.5 / 45 / 90 / 180 / 360 $\mu\text{g L}^{-1}$ and 0 / 0.25 / 0.5 / 1.0 / 2.0 / 4.0 $\mu\text{g L}^{-1}$ for the commercial formulations Tebuc 430SC (fungicide) and Imidagold 700WG (insecticide), respectively. To estimate growth rates, individuals were measured individually at the beginning and end of the experiment, on a hollowed-out slide under a stereomicroscope with 50x magnification, being handled with Pasteur pipettes. The effects were analyzed using Generalized Linear Models (GLM). The results showed that the growth rates for tebuconazole were 28.92 ± 3.87 ; 26.42 ± 4.76 ; 28.92 ± 5.80 ; 27.85 ± 4.51 ; 25.00 ± 5.66 and 23.92 ± 4.81 $\mu\text{m/day}$, while for imidacloprid the rates were 28.92 ± 3.87 ; 31.07 ± 4.43 ; 29.28 ± 4.25 ; 26.07 ± 5.16 ; 25.71 ± 2.85 and 20.00 ± 2.64 $\mu\text{m/day}$. Both pesticides caused a significant decrease ($p < 0.05$) in the growth of *S. rondoniensis* individuals at the highest concentrations. The change in the growth pattern of *S. rondoniensis* individuals is worrying, since studies show the occurrence of high concentrations of both pesticides in Brazil, ranging from 2,579 to 90.7 $\mu\text{g L}^{-1}$ for imidacloprid and up to 180 $\mu\text{g L}^{-1}$ for tebuconazole. Therefore, it is essential to strengthen public policies aimed at environmental monitoring, aiming to deepen the understanding of the real effects caused by the widespread occurrence of pesticides in aquatic ecosystems. This research was supported by the São Paulo Research Foundation - FAPESP (grant no. 2022/12104-4). Diego Ferreira Gomes and Thandy Junio da Silva Pinto have a postdoctoral scholarship supported by FAPESP (grant no. 2022/14293-9 and 2024/08039-8). David Silva Alexandre has a doctoral scholarship supported by FAPESP (grant no. 2024/00791-2).

2.03.P-We055 Imidacloprid: Spatial Avoidance and Behavioral Impacts on the Organism *Poecilia reticulata*

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Imidacloprid is a neonicotinoid insecticide widely used in agriculture due to its high efficiency in controlling insects that cause damage to various types of crops. This pesticide has been found in freshwater ecosystems worldwide, including Brazilian water bodies. The present study evaluated the spatial avoidance and behavioral impacts of the commercial formulation of imidacloprid (ICF) Galeão® on the Brazilian non-target aquatic organism *Poecilia reticulata* in an ecologically relevant concentration range. The Multi-Habitat Assay System (HeMHAS) was selected for avoidance and colonization tests with the organism *Poecilia reticulata*. The system was constructed with PVC boxes, formed by 12 square compartments (arranged in 3 columns and 4 rows), with a volume of 900 mL in each chamber, interconnected by 2 cm tubes allowing the exposure of the organisms to a concentration gradient of a chemical substance and free movement of the organisms between them. In this study, the length, width and height of the HeMHAS used were 1000 mm, 1000 mm and 100 mm, respectively. After filling the chambers (900 mL with different concentrations of the compound), 3 fish were inserted into each of the 4 compartments, totaling 12 fish per system. The tests were carried out for 90 min, under natural light conditions and were filmed with a suspended camera, avoiding interference from the human presence. The distribution of *P. reticulata* fish in the control occurred randomly, demonstrating that there was no interference from the system or the location on the organisms ($p > 0.05$). When exposed to imidacloprid, a significant difference was observed between the control and the 3 treatments with the compound ($p > 0.05$). The percentage data of the *P. reticulata* population within the system showed a clear tendency for the organisms to move to the less contaminated regions. It was found that after the 90-minute exposure, more than 70% of the population was concentrated between the control and the lowest concentration of imidacloprid, demonstrating that the compound has the potential to cause repellency in the organisms, directly interfering in the habitat selection process, and may lead to a great risk of loss of biodiversity of aquatic organisms. The authors thank to CNPq for financial support.

2.03.P-We057 Acute Toxicity of the *Bacillus thuringiensis* Commercial Bio-Insecticide for Aquatic Invertebrates

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The use of biopesticides has been increasing annually and worldwide. Their formulations derived from natural sources such as bacteria, animals, plants, or minerals. Today, about 90% of microbial biopesticides are derived from *Bacillus thuringiensis* (Bt). During sporulation, Bt strains synthesize crystal (Cry) and cytolytic (Cyt) toxic proteins, which have potential be used as pesticides in several crops. Insecticides containing *B. thuringiensis* are considered safe bioproducts and are commonly used in organic agriculture. Studies on Bt toxicity to non-target organisms have demonstrated that some commercial products were not

toxic, but some were, especially showing sublethal effects. Data from literature also suggest a synergy effect between the formulation compounds (spores, cleaved toxins, additives), which might increase the toxicity. Some additives used in commercial formulations might be even be more harmful than the active ingredients. Therefore, the aim of this work was to evaluate the acute toxicity of a commercial formulation from Bt in the freshwater microcrustean *Daphnia similis* and the marine amphipod *Parhyale hawaiiensis*. Bt (HizoBio) acquired from the market claimed to contain water, non-toxic additives and 5×10^8 CFU/mL of Bt. Aiming to also evaluate the effect of the formulation, we centrifuged the product 3 times for 20min at 2500rpm to remove Bt spores. After the last centrifugation, no pellet formation was observed. Acute toxicity tests were performed for the commercial product and the supernatant. Test with *D. similis* was carried out for 48h with photoperiod of 16:8h at 22°C in concentrations ranging from 0 to 0.1%. Test with *P. hawaiiensis* was performed for 96h with photoperiod of 12:12h at 24°C in concentrations ranging from 0 to 10%. Data was analyzed applying generalized logistic models. Bt was toxic to *D. similis* (EC50 0.014%) and *P. hawaiiensis* (LC50 0.37%). The supernatant was responsible for the toxicity detected in both organisms (EC50 0.012% and LC50 0.38%). Thus, we believe the toxicity is due to the formulation and not to the Bt spores. We aim to test more commercial compounds to evaluate the effect that the formulation/additives might cause in these aquatic species. The authors thank FAPESP (2022/04482-9)

2.03.P-We058 Ecotoxicological Sensitivity Mapping of the Province of Buenos Aires to Agrochemical Use

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The Province of Buenos Aires is one of the most agriculturally productive regions in Argentina. Dominated by the cultivation of genetically modified soybeans, corn, and wheat, among other crops, it exerts constant environmental pressure due to the use and application of pesticides, growth enhancers, and fertilizers. The dominant aquatic environments are shallow lagoons and low-flow streams, which are often temporary. There is a gradient in the physical and chemical conditions across the province's different watersheds. This study focuses on the variation in suspended solids concentration and salinity/conductivity and how these factors can influence the capacity to buffer the impact of agrochemicals. Two widely used pesticides were selected for analysis: those containing chlorpyrifos and glyphosate-based herbicides. Ecotoxicity was evaluated on algae and cladocerans using water samples from various environments within the Province of Buenos Aires. For this purpose, the province was divided into four sectors, and the watersheds associated with these sectors were sampled over a year. An ecotoxicological sensitivity map was created, taking into account the results of the bioassays and the gradients of conductivity and suspended solids.

2.03.P-We059 Pesticide Residues in Bio-Based Fertilizer Products – Assessing Product Safety by Bioassays

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The increased use and development of bio-based fertilizer (BBF) products supports the circular bioeconomy and sustainable food production by reducing dependency on mineral fertilizers and enhancing food security. However, fertilizer product raw materials may contain pesticide residues, such as pyralids (e.g., clopyralid and aminopyralid) and glyphosate. Pyralids can significantly inhibit plant growth even at very low concentrations, with sensitivity varying between plant species. Pyralids in crop residues that are further processed into fertilizers or growing media may eventually pose negative implications to crops or soil biota. Glyphosate, in turn, when introduced through feeding on fodder crops with glyphosate residues, can accumulate in manure, leading to reduced plant growth when manure is applied to fields. This study aims to promote the safety of BBFs. This includes increasing knowledge on BBF pesticide residue concentrations and the effects on plants and soil biota. In addition, the aim is to provide better tools for fertilizer product manufacturers and regulators to evaluate pesticide residue impacts on plants and ensure the safety of the fertilizer products, including growing media. The suitability of bioassays for assessing fertilizer product safety are evaluated by studying the effects of clopyralid and glyphosate on various plant species using e.g. standardized germination and shoot growth tests, and photosynthesis inhibition tests under controlled conditions. BBF products with known pesticide residue concentrations are also tested. Results from the study, expected in early 2025, will provide information that can be utilized in guidelines for the safe use of BBF products in agriculture and horticulture. This study is funded by Agri-food Research and Development funding of Ministry of Agriculture and Forestry of Finland (Makera)

2.03.P-We060 Non-target Arthropod Testing Under Extended Study Conditions: How to Handle Study Validity?

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Non-target arthropod (NTA) species on plants are used for the risk assessment of plant protection products. The required studies follow the test procedures for worst case laboratory studies according to guidelines to evaluate side-effects of plant protection products to non-target arthropods from Candolfi et al. (2000). These guidelines are related to eight arthropod species but according to ESCORT 2 workshop (2000) the parasitoid wasp species *Aphidius rhopalosiphii* and the predatory mite species *Typhlodromus pyri* were identified as sensitive indicator species in worst-case laboratory studies. One of the requirements on the validity of this study type is the use of a toxic standard substance for the verification that the respective arthropod species is sufficiently susceptible to potential effects of the test substance. Candolfi et al. (2002) present recommendations for the type and application rate of a toxic substance to be used in a worst-case laboratory test. However, this worst-case laboratory test design differs from natural conditions and is therefore often supplemented by studies under extended laboratory conditions, e.g. exposure to treated leaves, treated plants or aged residues on treated leaves and plants. Unfortunately, for these extended laboratory studies a reliable and conclusive information on the type and rate of a toxic standard is not generally available. This poster presentation compiles data on toxic substances from extended studies in the scientific literature and the publicly available draft assessment reports from registration dossiers and gives recommendations for different types of extended NTA studies.

2.03.P-We061 Evaluation of Triazole Fungicide Exposure To The Development of *Tenebrio molitor*
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The use of pesticides, in particular triazole fungicides, contributes significantly to biodiversity loss in agroecosystems. These fungicides affect fungal cell wall formation by inhibiting ergosterol synthesis, and in arthropods they can disrupt the production of ecdysteroids, key hormones in moulting and reproduction. Since invertebrates are essential for ecosystem balance, this study evaluated the effects of triazoles on the mealworm (*Tenebrio molitor*) by simulating exposure scenarios through the ingestion of cereal seeds treated with prothioconazole and tebuconazole. Physiological variables (size, weight and development) and changes in gene expression linked to ecdysteroidogenesis were measured. Three exposure treatments were carried out: seeds treated with the labelled dose of Raxil® Plus, 50:50 and 25:75 mixtures of treated and untreated seeds, and a control with untreated seeds. Sixty larvae per treatment were exposed for 10 days, ingesting only the allocated seeds. Subsequently, they were offered oats, wheat bran and vegetables, and their development was monitored until the adult stage. Weight, mortality, malformations and development time were recorded. For molecular analysis, total RNA was extracted from larvae, pupae and adults and converted into cDNA for qRT-PCR. Gene expression was assessed using specific primers and relative differences from the control were calculated. Mealworm showed rejection of treated seeds, especially in the intermediate treatment. A decrease in weight was also observed in the higher treatments. No significant differences in development time were observed. Gene analysis revealed a lower expression of the enzymes Spo, Sad, Shd and the transcription factor HR3 in the highest treatment. In the intermediate treatment, a reduction of Spo and HR3 was detected, as well as an overexpression of the regulator E75. These changes suggest that fungicides interact with key elements of the ecdysteroidogenesis pathway, which could compromise essential functions in exposed arthropods. In conclusion, exposure to triazole fungicides may affect both physiological parameters and genetic regulation of hormonal processes in *Tenebrio molitor*, highlighting the need to assess their impact on biodiversity and agroecosystem functioning.

2.03.P-We062 Assessing Seed Coating Pesticide Risk to Honey Bee (*Apis mellifera anatoliaca*) Populations: A Study on Toxicity, Residue Levels, and Ecotoxicological Implications in Turkish Maize Fields

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This study investigates the potential risks associated with thiamethoxam and clothianidin, neonicotinoid insecticides, on honey bees (*Apis mellifera anatoliaca*) in Turkey. The research focuses on honey bees foraging water from guttation fluid, exploring the toxicity of clothianidin and thiamethoxam, and assessing their residue levels in guttation fluid from maize plants. Ecotoxicological tests were conducted to evaluate the impact of guttation fluid on honey bee populations. In bee toxicity tests with guttation fluid collected from plants grown from seeds treated with thiamethoxam, one of the neonicotinoid insecticides, 80% mortality was observed as early as 4 hours during the first 5 days of guttation fluid. In the following days, the mortality rate initially decreased but remained above 50% until the 10th day, with fluctuations

observed. Counts made at 24 and 48 hours of the trials indicated that all the bees in the trials died by the 9th day. Although guttation fluid collected on subsequent days showed an effect of about 50% at 48 hours, when the experiment was extended to 72 hours, gut fluid collected on all days caused more than 50% mortality. Thiamethoxam caused very high mortality from 24 h in the collected gut fluid trials, and at 72 h, it caused over 80% mortality on almost all days gut fluid was collected. Similar results were found in the experiments with clothianidin, which resulted in mortality rates between 80% and 100% during the experiments. Further research and regulatory measures are warranted to address and mitigate the risks posed by these pesticides to pollinator populations and the overall health of ecosystems.

2.03.P-We063 Nationwide Monitoring and Risk Assessment of Neonicotinoid Insecticides in Korean River Basins: A Species Sensitivity Distribution Approach

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Neonicotinoid insecticides (NNIs) are the most widely used insecticides globally, having replaced organophosphates, carbamates, and pyrethroids due to their higher selective toxicity to pests and lower impact on non-target organisms. However, concerns remain over their high toxicity to pollinators, particularly honeybees, which can disrupt entire ecosystems. This study presents a comprehensive assessment of NNIs in Korean river basins, focusing on their ecological risks and spatial-temporal occurrences. Nationwide monitoring was conducted across four major river basins in Korea. Results indicated that southern basins, such as Yeongsan and Nakdong, exhibited significantly higher NNI concentrations, likely due to extensive agricultural activities. Seasonal variations revealed peak NNI levels during application periods, with significant runoff-driven transport into aquatic ecosystems. Risk assessments were performed using Species Sensitivity Distributions (SSDs), suggesting the sensitivity of aquatic species to NNIs, particularly invertebrates. The SSD-derived Predicted No Effect Concentration (PNEC) values were compared with conventional standards, showing the conservative assessment of SSD-based risk assessment. Our findings emphasize the need for tailored regulatory approaches in managing NNIs in agriculture-intensive regions and urban areas, and indicate the importance of accounting for transformation products in risk assessments. This research contributes to the development of a refined NNI monitoring paradigm, addressing both acute and chronic ecological risks.

2.03.P-We064 Wellness Peeling? Reduced Exposure to Pesticides via Dehusking in Small Mammals

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Dehusking of seeds before consumption is a well-known behaviour in small granivorous mammals. This behaviour strongly influences the potential uptake of pesticides, either used as seed treatments or applied via overspray on weed and crop seeds (on the plant or ground). In all cases, the greatest amount of pesticide residues is usually located on the outer husk of the seeds; therefore, dehusking can lead to significant exposure reduction for selected species. The recently noted revised EFSA Guidance Document on Risk Assessment for Birds and Mammals, which will be implemented in 2025, highlights dehusking as suitable refinement option and gives advice on specific parameters for conducting dehusking studies. We present an extensive data set obtained from a dehusking study with a cereal seed treatment product, providing data for wood mouse (*Apodemus sylvaticus*) as well as yellow-necked mouse (*A. flavicollis*), two common small mammal species known to regularly feed on seeds and both potential focal species in European pesticide risk assessment schemes. The study design followed a semi-field approach with wild-caught animals, which were individually housed in cages, and presented with treated cereal seeds. Dehusking behaviour was observed via video recordings. In addition, actual exposure reduction was quantified via residue analysis to enable derivation of an actual dehusking factor for risk assessment purposes. Our results clearly demonstrate extensive dehusking behaviour in both tested species leading to considerable exposure reduction. This substance-specific approach with a high sample size and a robust quantification method is thus in line with previous data for wood mouse generated in the lab and confirms the suitability of the study setup. In addition, we hereby provide important data for yellow-necked mouse as potential focal species and can demonstrate that exposure reduction via dehusking in this species is in a similar range as in wood mice. While the study design was crop- and substance-specific, the general methods can be adapted and applied for different scenarios or even species to derive dehusking factors, which is now in light of the new Bird and Mammal Guidance Document and the increased importance of granivorous scenarios of even greater importance as refinement option.

2.03.P-We065 Peel, eat, repeat - Monitoring of Bird Dehusking Behaviour

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Regardless of the application type (seed treatment on crop seeds or spray application on weed or crop

seeds), the bulk of pesticide residues ends up on the outside of the seed the husk. Thus, the removal of the seed husk prior to ingestion (so-called dehushing) leads to a considerable reduction of the exposure. The revised EFSA Guidance Document on Risk Assessment for Birds and Mammals, set to be implemented in 2025, identifies dehushing as a valuable refinement option and offers recommendations on the key parameters for conducting dehushing studies. In order to estimate the exposure for the use as a dehushing factor in avian risk assessments, it is important to quantify the extent and efficiency of the dehushing behaviour in addition to the analysis of which portion of active ingredients on seed is not ingested by a dehushing bird. Linnets (*Linaria cannabina*) and serins (*Serinus serinus*) are two granivorous bird species, widespread in many agricultural landscapes and the only two European bird species that regularly occur in farmland, which exhibit a nearly entirely granivorous diet. Here we present results of a study that aimed to investigate how linnets and serins handle and dehush six different kinds of weed and crop seeds and to additionally quantify the reduction of exposure to contaminated seeds via this behavioural trait. The study was conducted using a semi-field approach over two years, involving a large number of wild-caught birds, which were individually placed in cages at trapping sites and provided with treated seeds. As proposed by the new EFSA Guidance Document, a non-toxic substance (capsaicin) substituting the pesticide was used to measure the exposure reduction caused by dehushing. Our results support that the two bird species showed different preferences for the offered seed types and an upper limit of seed size included in common serin feed. We could also demonstrate that both species commonly dehush seeds prior to consumption. The handling of different seed types and the comparison between both species will be presented and discussed.

2.03.P-We066 Effects of Subchronic Exposure to Pyriproxyfen and Fipronil in Zebrafish (*Danio rerio*): Impacts on Thyroidal and Gonadal Axis Disruption and Oxidative Stress Gene Expression

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Pyriproxyfen, widely used to combat vectors like dengue mosquitoes, and Fipronil, an insecticide and acaricide applied against various pests, are essential tools in pest control. However, their growing use raises concerns about potential impacts on human health, particularly on the endocrine system. Even at low doses, these compounds can mimic hormones, disrupting target organs and interfering with critical physiological functions. To evaluate the effects of chronic exposure to Pyriproxyfen (PPF) and Fipronil (FPN) on endocrine disruption, growth, and oxidative stress in the brain of zebrafish (*Danio rerio*). Zebrafish were allocated to eight aquariums (n=8 per group) and divided into groups: saline control (CS), DMSO control (CD), and exposure to PPF at 0.01, 0.1, 0.5, 1.0, and 1.5 µg/L or FPN at 0.01, 0.1, 1.0, 10, and 50 µg/L for 15 days. Brain and thyroid tissues were collected for gene expression analysis related to the thyroidal and gonadal axes, as well as oxidative stress markers, using real-time PCR. Data were analyzed with one-way ANOVA and Dunnett's multiple comparison test, with significance set at $p < 0.05$. Endocrine Disruption: PPF exposure at 0.5 and 1.2 µg/L significantly increased tsh gene expression, while FPN exposure at 1.0 and 50 µg/L showed similar effects. FPN also elevated tsh, lh, fsh, kisspeptin expression at 0.01 and 1.0, 1.5 µg/L, suggesting hypothyroidism. Both compounds reduced gh expression across multiple concentrations, likely linked to thyroid hormone activity. Antioxidant enzyme genes (cat, sod) were upregulated at 0.01 µg/L PPF exposure, while sod and gpx showed increased expression at 0.01 and 0.1 µg/L FPN, respectively. Higher FPN concentrations (10 and 50 µg/L) significantly upregulated cat expression. Dio2 expression increased with 0.5 and 1.2 µg/L PPF and 0.1 µg/L FPN exposure. PPF at 0.5 µg/L also upregulated tra expression. These findings suggest an adaptive response to oxidative damage caused by these insecticides. Chronic low-dose exposure to PPF and FPN caused thyroid and gonadal endocrine disruption in zebrafish, evidenced by increased expression of tsh, gnrlh, kisspeptin, lh, and fsh, alongside reduced gh expression. Both insecticides also induced oxidative stress in the brain, marked by upregulation of antioxidant enzymes (cat, sod, and gpx). This suggests that brain tissues attempt to counteract oxidative damage caused by these compounds, highlighting their potential as endocrine disruptors. FAPESP Project: 2022/03094-5

2.03.P-We067 Toxic Response of *Danio rerio* Exposed to Two Commercial Formulations of Glyphosate

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Agriculture is the largest production activity consumer of pesticides. Glyphosate is the most consumed herbicide used to protect several crops (soybean, corn, cotton, wheat, fruits, and vegetables) from weeds and is coupled with transgenic crops. This herbicide is known for its effectiveness, accessibility, low cost, and ease of use. In Mexico, it is sold under various brand names (AquaMaster®, Faena®, and Mamba®); these formulations differ in composition, but all contain surfactants to allow even distribution on target

species. Because of its liquid condition, glyphosate can easily be washed into waterbodies, leading to pollution and potential damage to non-target aquatic biota, including protozoa, microalgae, amphibians, insects, and fish. The present study aimed to assess the toxic effects of two glyphosate commercial formulations (Faena Classic® and Faena Fuerte®) on the early life stages of zebrafish (*Danio rerio*). Bioassays were conducted following OECD protocol 236 to assess the toxic effects. The embryos were exposed to varying concentrations of the formulations for 144 hpf. Glyphosate levels in the formulations were quantified using an ELISA immunoassay. The median lethal concentration (LC50) of each formulation was determined, with Faena Fuerte® showing higher toxicity (LC50: 3.78 mg/L) compared to Faena Classic® (LC50: 4.18 mg/L). In addition to higher mortality, Faena Fuerte® also caused more pronounced sublethal effects, such as coagulation (40%), edema (10%), and scoliosis (15%). The results indicated that Faena Fuerte® is more toxic to zebrafish embryos than Faena Classic®. The quantification of glyphosate showed that both herbicides have 328 g/L of glyphosate in their formulation; however, additives such as emulsifiers, solvents, and surfactants (surfactants) may be different, and these modified the toxicity of the active ingredient. This study reveals that both herbicides produced lethal and sublethal toxic effects in aquatic organisms, so it is essential to establish controls and implement measures to prevent these compounds from contaminating the aquatic environment. Also, more attention and regulation must be given to the inert ingredients in formulations, as they can increase toxicity to non-target species.

2.03.P-We068 Pesticide Mixture Effects on Bioaccumulation and Toxicity in Biofilms

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Pesticides threaten aquatic ecosystems, particularly biofilm communities (microalgae, bacteria, and fungi within an extracellular matrix), which underpin food webs and act as sensitive water quality indicators. Pesticides' toxicity and bioaccumulation in biofilms depend on properties like logK_{ow} and mode of action, yet studies often overlook the effects of pesticide mixtures, common in the environment. Moreover, bioaccumulation doesn't always correlate linearly with exposure, limiting concentration-based risk assessments. Understanding mixture effects whether competitive, additive, or synergistic is critical as pesticide pollution in aquatic systems continues to rise. This study aims to develop a predictive model to evaluate the impact of pesticide mixtures on bioaccumulation and toxicity in biofilms, based on pesticide properties. Based on existing literature, we hypothesize that pesticides sharing the same mode of action will exhibit additive effects, whereas those with different modes of action may result in synergistic effects, as they target different receptors. To achieve this, we will conduct an experiment exposing biofilms, composed of the picocyanobacterium *Synechococcus bacillaris* and the diatom *Phaeodactylum tricorutum*, to various pesticide mixtures and assessing pesticide bioaccumulation and toxicity. Five pesticides diuron, terbuthylazine, tebuconazole, acetochlor, and hexazinone were selected for their diverse and/or similar physicochemical properties. Initially, biofilms will be exposed to each pesticide individually to determine their EC50 values, which will be used to establish the equivalent of 1 Toxic Unit (TU) for each compound. The single-exposure experiment will be used to develop an initial model linking pesticide concentration in water and pesticide properties, with bioaccumulation and toxic effects such as photosynthesis inhibition. Binary mixture exposure data will then be incorporated to account for mixture effects, refining the model further. Subsequently, biofilms will be exposed to all possible binary mixtures, with each pesticide at 1 TU, to evaluate the mixture effect. Pesticide concentrations will be measured in both the medium and the biofilm, while toxicity will be assessed by measuring photosynthesis inhibition in the biofilm. These efforts reveal the complexity of pesticide bioaccumulation, where non-linear relationships make concentration alone an insufficient risk indicator. This project is funded by FORMAS (grant number : 2023-01095)

2.03.P-We069 Histopathological Effects of Commercial Diflubenzuron Formulations on Wild-Type Zebrafish (*Danio rerio*): A Sub-Chronic Exposure Study

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In aquatic ecosystems, pollutants like insecticides pose risks to wildlife. Diflubenzuron (DFB), used in agriculture and veterinary practices, may harm non-target species. Despite the widespread application of its commercial formulations, research addressing their toxicity and morphological effects on non-target

species remains limited, leaving a comprehensive understanding of their impacts unresolved. Our objective was to assess histopathological alterations in the liver, kidney, and gonads of adult male and female wild-type zebrafish (*Danio rerio*) following a sub-chronic (14-day) exposure to two commercial DFB formulations, one for agriculture and the other for veterinary use. Ten specimens (?=5; ?=5) of zebrafish per treatment were exposed to six different DFB formulations concentrations (DBF concentrations ranging from 0.025-1.00 mg/L). Dimethyl sulfoxide (DMSO) was used as intermediate solvent. The experimental design included a negative and a solvent control group. Samples were hematoxylin-eosin stained, scanned, and analyzed via Aperio ImageScope 12.4.6 (20x). Quantitative analyses used GraphPad Prism version 10.0.0. Regarding the liver, telangiectasia and peripheral nuclei occurred in all treatment groups, with pronounced severity at 0.125-0.5 mg/L. Steatosis, altered hepatocyte nuclear morphology, and fibrosis were most evident at 0.065-0.25 mg/L treatments. For the kidney, all exposed specimens exhibited progressive dilation of the capillaries, and degeneration of the distal and proximal tubules and glomeruli, often accompanied by a reduction in Bowman's space. Moreover, extravasation of inflammatory exudate primarily occurred in specimens exposed to the highest concentrations of veterinary formulation. All treated males exhibited spermatogonia, spermatocytes, spermatids, and spermatozoa, with regions of pyknosis observed starting at 0.065 mg/L. Concerning the ovaries, most of the treated females showed the five stages of maturation (primary growth, pre-vitellogenic, early vitellogenic, mid-vitellogenic, and complete vitellogenesis), although no significant differences occurred compared to the control group. Fused vitellogenin granules appeared even at 0.025 mg/L. Furthermore, necrotic areas appeared in all treatment groups across the three focal organs. The findings demonstrate that commercial DFB formulations cause morphological damage to adult zebrafish, with veterinary formulation exhibiting itself as the most toxic. This work was funded by the São Paulo Research Foundation (FAPESP) under grant numbers 22/03094-5 (Zebratox Project: Toxicological Screening of Larvicides of Relevance to Public Health and Veterinary Medicine), 23/15540-2, 23/09884-0, 2024/10993-1 and 24/01418-3, and the National Council for Scientific and Technological Development (CNPq), Brazil (grant number 153725/2024-9).

2.03.P-We070 Histopathological Effects Caused by Chronic Exposure to the Insecticide Diflubenzuron in Zebrafish

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Vector-borne neglected tropical diseases (NTDs), such as dengue, chikungunya, yellow fever, and Zika, are major public health concerns. Diflubenzuron (DFB), a larvicide that inhibits chitin synthesis, is recommended by the World Health Organization for mosquito control in specific contexts, including drinking water reservoirs at concentrations up to 0.25 mg/L. Despite its reported safety for non-target organisms, data on the chronic environmental toxic effects of DFB remain limited. This study used zebrafish (*Danio rerio*) as a toxicity model to evaluate DFB chronic exposure effects. All procedures were approved by the Ethics Committee for the Use of Animals (CEUA) of the Federal University of São Paulo (approval number 5220291 122). Wild-type zebrafish were exposed to six DFB concentrations (0.025 0.75 mg/L), a negative control, and a solvent control for 90 days. Histopathological analyses focused on the liver, intestine, and kidney. Chronic exposure to DFB caused significant liver tissue alterations, including peripheral nuclear displacement, fibrosis, hepatocyte dilation, and necrosis in the liver, with effects observed at concentrations as low as 0.025 mg/L. Chronic exposure to DFB resulted in a concentration-dependent reduction in hepatocyte nuclear area. At 0.025 mg/L, the average reduction was 2.9 μm^2 compared to the control, reaching 5 μm^2 after exposure to 0.75 mg/L for 90 days ($p < 0.05$). Renal alterations, such as reduced Bowman's capsule space and glomerular degeneration, were prominent at higher concentrations (? 0.25 mg/L). In the intestine, DFB exposure stimulated mucus production, goblet cell proliferation, and structural damage, including villus lysis and epithelial detachment. These findings demonstrate the potential of DFB to induce severe damage to multiple organs, even at low concentrations. The study underscores the need for further research on DFB's long-term ecotoxicological impacts and highlights the zebrafish as a valuable model for ecotoxicological studies. Integrating these insights into NTDs control strategies may help minimize environmental health risks. This work was supported by São Paulo Research Foundation (FAPESP) (grant numbers 2022/03094-5 Bruno Fiorelini, 2024/10993-1 and 2024/01418-3 Flavio Tominaga, 2023/09884-0 Rafaella Brito, 2023/15540-2 Amanda Valle)

2.04 Mechanistic Insights, Advances, and Challenges in Behavioral Ecotoxicology and Neurotoxicity Assessment

2.04.T-01 Pharmaceutical Pollution Alters River-to-Sea Migration Success in Atlantic Salmon (*Salmo salar*)

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Despite the growing threat of pharmaceutical pollution, we lack an understanding of whether and how such pollutants influence animal behaviour in the wild. Using laboratory- and field-based experiments across multiple years in Atlantic salmon (*Salmo salar*; n = 730), we demonstrate that the globally detected anxiolytic pollutant clobazam accumulates in the brain of exposed fish and alters river-to-sea migration success. Clobazam exposure increased the speed with which fish passed through two hydropower dams along their migration route, resulting in greater migration success compared to controls. We found that such effects may arise from altered shoaling behaviour in fish exposed to clobazam. Drug-induced behavioural changes, even when seemingly beneficial, are expected to have wide-ranging consequences for the ecology and evolution of wild populations.

2.04.T-02 Mechanism of Ocular Toxicity of Antidepressants in Zebrafish

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Global use of antidepressants is steadily increasing, with incomplete removal during wastewater treatment raising concerns about risks to aquatic ecosystems. Antidepressants act on the neuronal system by affecting neurotransmitter levels and have shown to cause behavioural effects in aquatic organisms. While behavioural effects are often attributed directly to altered neurotransmitter levels, there is a knowledge gap regarding whether effects on the visual system also contribute to these effects. This work aims to assess adverse effects of antidepressants on the visual system of early life stage zebrafish and to determine the underlying mechanism. Embryos were exposed to sublethal concentrations of the tricyclic antidepressants amitriptyline (AMI) and nortriptyline (NOR) and the selective serotonin reuptake inhibitor sertraline (SER). Effects to visual function were assessed via the optokinetic response (OKR) assay, cellular structure of the eye via histology, and gene expression via mRNA sequencing and qPCR. The OKR assay, which measures eye movements in reaction to an optical stimulus, showed significant effects for 4.99 and 234 µg/L of AMI and 20.7 µg/L of NOR (measured concentrations), with zebrafish embryos exhibiting 24%, 83% and 60% less eye saccades compared to the solvent control (0.01% DMSO) respectively. Histology revealed a significant increase in retinal pigment epithelium (RPE) thickness for both the 234 µg/L AMI (13.34 µm) and 20.7 µg/L NOR (13.29 µm) groups, compared to the solvent control (11.52 µm). Transcriptomics revealed significant effects to processes including synaptic signalling, neuron morphogenesis, and visual perception. Among the differentially expressed genes associated with visual perception, several genes involved in photoreception (*opn1mw1*, *rho*) and vision (*arr3a*, *pde6c*) were dysregulated. OKR assay with SER showed a significant decrease in eye saccades compared to the control, 34% and 86% respectively, at 100 and 1000 µg/L (nominal). Histology revealed a significant decrease by 17% in RPE thickness for 1000 µg/L SER. The results provide evidence that antidepressants can induce ocular toxicity in early life stage zebrafish on multiple levels of biological organization. While serotonin is implied to be involved in the mechanism, current experiments with the transgenic zebrafish line tg(tg:mCherry) focus on testing the hypothesis that thyroid disruption is the mechanism connecting altered serotonin levels to ocular toxicity. The authors would like to thank the European Commission and the Ministry of University and Research (MUR, Italy), the Federal Ministry of Education and Research (BMBF, Germany), the Research Council of Norway (RCN, Norway) and State Research Agency (AEI, Spain) for funding in the frame of the collaborative international consortium PHARMASEA financed under the 2020 AquaticPollutants Joint call of the AquaticPollutants ERA-NET Cofund (GA N° 869178). This ERA-NET is an integral part of the activities developed by the Water, Oceans and AMR JPIs. The PhD grant of Marwin Jafari is funded by the University of Stavanger (Norway).

2.04.T-03 Early Life Exposure to Endocrine Disruptors: Understanding the Neuroendocrine Impact Using Metabolome Mapping of Rat Models

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Exposure to endocrine-disrupting chemicals (EDCs) in humans during pregnancy and early stages of life can impair normal brain development and reproductive function patterns, leading to severe pathologies later in life. Studies have shown developmental neurotoxicity effects of bisphenols (BPA mostly) and phthalates. However, for many chemicals, such as pyrethroids, per- and polyfluoroalkyl substances (PFAS), organophosphate flame retardants, and plasticizers, hardly any information is available on the relation between endocrine disruption (ED) and potential neurodevelopmental (DNT) effects. To better understand the link between ED and DNT outcomes, rats (F0 rat dams) were exposed to six EDCs (BPF, BBzP, DINCH, PFOS, permethrin (PMT), triphenyl phosphate (TPHP)) from pre-mating until lactation. One pup/sex/litter was raised to adulthood for testing of activity, learning and memory, anxiety and social behavior. Targeted (steroid hormones, thyroid hormones, neurotransmitters) metabolomics and untargeted lipidomics of hippocampus (PND6) samples were used to map the metabolic pathways affected by these six EDCs and link these to the memory function. Changes in specific neurosteroids, neurotransmitters and hundreds of lipids in developing rat hippocampus were sex- and chemical-specific and correlated to adverse outcomes on memory of adult rat littermates. Memory function (Morris water maze) was impaired in adult male offspring by BPF and BBzP, and in females by DINCH and TPHP. Steroids, neurotransmitters and lipids levels in hippocampus (PND 6) correlated with sex-dependent behavioral outcomes. For instance in females pregnenolone-sulphate (PREG-S), GABA, and in males PREG-S, acetylcholine, histamine and corticosterone, and in both sexes hundreds of lipids were significantly regulated in the adverse memory function group compared to the control. Previously studies demonstrated a significant correlation between PREG-S and cognitive function, and also a relation between PREG-S and the neurotransmitter acetylcholine. Altered lipid levels in hippocampus (PND 6) by EDCs correlated with sex-dependent behavioral outcomes; lipids were mainly upregulated in females and mainly downregulated in males. Our findings will primarily be useful to understand the molecular mechanism of EDC toxicity in the developing neuroendocrine system, to elucidate the relationships with behaviour and memory function, and provide potential biomarkers for ED-linked DNT. This project received funding from EU Horizon 2020 (No. 825759, ENDpoiNTs).

2.04.T-04 The Application of Mass Spectrometry Imaging to Study Neurodevelopment and its Disruption by PFOS in a Tadpole Model

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Mass spectrometry imaging (MSI) is an exciting emerging technology that adds spatial information to mass spectrometry analysis, allowing mapping of specific biomolecules in the tissue. This is particularly interesting in the brain, where different tissue regions have distinct functions, and in sensory organs such as the olfactory system. Here we utilized a representative of true frogs, the cosmopolitan tadpole model American bullfrog (*Rana [Lithobates] catesbeiana*), and MSI to first construct a brain developmental atlas throughout metamorphosis. Then we used this baseline to study neurodevelopmental disruption due to PFOS exposure. Premetamorphic tadpoles were exposed to PFOS in a dose-range (0-100 µg/L) for 48 h. Tadpole heads were sampled for MSI and blood and brain samples were also analyzed with a complementary 4D lipidomics technique (LC-TIMS-MS/MS) using the same mass analyser. Along with the effects of toxicant exposure, the MSI also showed the tissue localization of PFOS which localized to a specific neuroendocrine gland and to the olfactory epithelium. Hence, the present study gives unprecedented information about tissue distribution of a legacy contaminant, as well as tissue specific effects. The possibility to link the results to a developmental baseline allows us to also evaluate the biological significance of affected biomolecules and suggest new biomarkers for assessment of developmental neurotoxicity.

2.04.P-Tu173 Environmentally Relevant Concentrations Of Seleno-Methionine Impair Neurodevelopment and Behaviour in Larval Zebrafish (*Danio rerio*)

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Selenium (Se) is an essential micronutrient to fishes; however, it becomes extremely toxic when its concentration rises slightly above the physiological optimum. In contaminated systems, fishes accumulate Se primarily as seleno-methionine (Se-Met). While the detrimental effects of Se-Met in adult fishes are well characterized, the embryotoxicity of Se-Met in fishes remains poorly understood beyond the teratogenic effects. With that in mind, zebrafish embryos (2-hour post fertilization, hpf) were exposed to environmentally relevant concentrations of Se-Met (0, 5, 10, and 25 $\mu\text{g/L}$) until 120 hpf. We found that exposure to Se-Met (10 and 25 $\mu\text{g/L}$) induced significantly higher larval mortality and deformity rates compared to the control. In addition, our study also indicated that exposure to Se-Met (5 and/or 10 $\mu\text{g/L}$) impaired thigmotactic and reflexive behaviours in zebrafish larvae. Furthermore, Se-Met exposure induced a dose dependent increase in reactive oxygen species (ROS) generation and apoptosis, dysregulation of proteins and genes involved in the dopaminergic, serotonergic, and cholinergic signaling pathways, and disrupted neurogenesis in larval zebrafish. Overall, our findings provide important new insights into the neuro-behavioural effects of Se-Met exposure in embryonic zebrafish, further highlighting

the risks by selenium contamination in aquatic ecosystems. Md Helal Uddin was supported by the Dean's Graduate Scholarship of the University of Saskatchewan. This work was supported by the discovery grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to Douglas P Chivers and Som Niyogi.

2.04.P-Tu175 High-Content Screening in *C. elegans* for Neuronanosafety: Impacts of Silver Nanoparticles on Neurodegeneration

Nivedita Chatterjee¹, Marie Pierron², Laurent Mouchiroud² and Ernesto Alfaro-Moreno¹, (1)International Iberian Nanotechnology Laboratory, Portugal, (2)Nagi Bioscience, Switzerland

With the increasing production and use of nanomaterials, concerns about their neurotoxic potential have risen, prompting investigations into their effects on neuronal health. *Caenorhabditis elegans* (*C. elegans*), with its fully mapped nervous system and conserved genes, serves as an excellent model for studying neurotoxicity due to its genetic tractability and rapid neurobehavioral assessments. As a New Approach Methodology (NAM) tool-kit, it provides a cost-effective, ethical alternative to traditional animal models, enabling high-content screening and nanosafety research. This study evaluates the neurobehavioral and neurodegenerative effects of silver nanoparticles (AgNPs) on *C. elegans* using the high-content screening platform SydLab™ One from Nagi Bioscience. Significant growth inhibition and developmental delays were observed at higher concentrations (0.5 and 5 $\mu\text{g/mL}$) of AgNPs, with a mean growth (area) rate of $2.56 \pm 0.49 \mu\text{m}^2/\text{hour}$ for controls, compared to 1.45 ± 0.28 for 0.5 $\mu\text{g/mL}$. Reproductive assessments showed reduced progeny accumulation, with a mean rate of 2.4 ± 1.75 progeny/hour at 0.5 $\mu\text{g/mL}$ compared to 4.15 ± 1.77 for controls, and no progeny observed at 5 $\mu\text{g/mL}$ until 80 hours, indicating reproductive failure at highest concentrations. Additionally, we investigated the differential neurobehavioral and neurodegenerative effects of AgNPs (0.05 $\mu\text{g/mL}$) across wild-type and neurodegenerative disease models (Alzheimer's disease model, GMC101 and Parkinson's disease model, NL5901) over 11 days. Survival (% viability) and behavioral endpoints, including mean body bending frequency (Hz) and locomotion speed (mm/sec), indicated increased sensitivity to AgNPs in the Alzheimer's disease model (GMC101). In contrast, the Parkinson's disease model (NL5901) showed no significant differential effect. This increased sensitivity in the Alzheimer's model was supported by glutamatergic neurodegeneration observed in the reporter strain (DA1240). These findings suggest that AgNPs exposure may be associated with amyloid-beta aggregation, potentially amplifying Alzheimer's disease risk. Overall, *C. elegans* proves to be a valuable model for high-content neurobehavioral screening, offering predictive insights into nanoparticle effects on vulnerable populations, making it an ideal tool for NAM in neurotoxicology and nanosafety research. This study was funded by the European Union's H2020 projects, Sinfonia (N.857253), LEARN (N.101057510) and iCare (N.101092971).

2.04.P-Tu178 Neurobehavioural Effects of Bisphenol S on the Early Life Stages in Zebrafish (*Danio rerio*)

A.K.M. Munzurul Hasan, Mahesh Rachamalla, Sravan Kumar Putnala, Md Helal Uddin, Som Niyogi and Douglas P. Chivers, Department of Biology, University of Saskatchewan, Canada

Bisphenol S (BPS) is a widely used synthetic compound known as an endocrine-disrupting chemical (EDC). It is commonly found in epoxy resins and polycarbonate plastics, materials frequently used for food storage containers and baby bottles. The ability of BPS to bind predominantly to estrogen receptors raises significant concern, as it can interfere with different neurological functions leading to neurobehavioural deficits. Despite extensive research documenting various adverse effects of BPS in adult fish, its neurobehavioural effects, especially in early life stages of fish, remain poorly understood. In the

present study, zebrafish embryos (4-hour post fertilization, hpf) were exposed to environmentally relevant concentration of BPS (vehicle control, 0 and 30 µg/l) until 120 hpf. Subsequently, behavioural effects of BPS were evaluated by examining various behavioural responses including thigmotaxis (120 hpf), reflexive movement (120 hpf), social preference (21 dpf (day post fertilization)), and object recognition (26 dpf). These results indicated that exposure to BPS impaired thigmotaxis, reflexive movement, social and object recognition behaviours in zebrafish larvae. In addition, reactive oxygen species (ROS) production and apoptosis were evaluated at 120 hpf zebrafish larvae, which were found to be elevated following exposure to BPS. Moreover, gene expression analysis at 120 hpf indicated that BPS exposure resulted in the dysregulation of genes involved in serotonergic (slc6a4a, slc6a4b, tph2, htr1aa, htr1ab, htr1b, htr1d, htr2aa, htr2b) and cholinergic (ache, chata, creb1a) neurotransmitter pathways, apoptosis pathway (caspase-3, p-53), oxidative stress response (gpx, mn-sod), and neuroinflammation (il-1?, il-6). These findings suggest that BPS induces oxidative stress and apoptosis, which likely leads to disruption of neural development and signaling pathways involved in regulating behavioural responses. Overall, our study highlights that developmental exposure to BPS can induce marked behavioural deficits in larval zebrafish, and provides new insights into the neurotoxic mechanisms underlying its behavioural effects.

2.04.P Mechanistic Insights, Advances, and Challenges in Behavioral Ecotoxicology and Neurotoxicity Assessment

2.04.P-Tu172 EthoCRED: A Framework to Guide Reporting and Evaluation of the Relevance and Reliability of Behavioural Ecotoxicity Studies

Michael Grant Bertram¹, **Marlene Ågerstrand²** and **Tomas Brodin¹**, (1)Wildlife, Fish and Environmental Studies, Swedish University of Agricultural Sciences, Sweden, (2)Stockholm University, Sweden

Behavioural analysis has been attracting significant attention as a broad indicator of sub-lethal toxicity and has secured a place as an important subdiscipline in ecotoxicology. Among the most notable characteristics of behavioural research, compared to other established approaches in sub-lethal ecotoxicology (e.g. reproductive and developmental bioassays), are the wide range of study designs being used and the diversity of endpoints considered. At the same time, environmental hazard and risk assessment, which underpins regulatory decisions to protect the environment from potentially harmful chemicals, often recommends that ecotoxicological data be produced following accepted and validated test guidelines. These guidelines typically do not address behavioural changes, meaning that these, often sensitive, effects are not represented in hazard and risk assessments. Here, we propose a new tool, the EthoCRED evaluation method, for assessing the relevance and reliability of behavioural ecotoxicity data, which considers the unique requirements and challenges encountered in this field. This method and accompanying reporting recommendations are designed to serve as an extension of the Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) project. As such, EthoCRED can both accommodate the wide array of experimental design approaches seen in behavioural ecotoxicology, and could be readily implemented into regulatory frameworks as deemed appropriate by policy makers of different jurisdictions to allow better integration of knowledge gained from behavioural testing into environmental protection. Furthermore, through our reporting recommendations, we aim to improve the reporting of behavioural studies in the peer-reviewed literature, and thereby increase their usefulness to inform chemical regulation.

2.04.P-Tu174 Mechanism of Ocular Toxicity of Antidepressants in Zebrafish

Marwin Jafari¹, **Jason Tyler Magnuson²**, **Katharina Brotzmann³**, **Sebastian Eilebrecht⁴**, **Fabian Essfeld⁵** and **Daniela Maria Pampanin¹**, (1)University of Stavanger, Department of Chemistry, Bioscience and Environmental Engineering, Norway, (2)U.S. Geological Survey, United States, (3)States3University of Heidelberg, Centre for Organismal Studies, Germany, (4)Ecotoxicogenomics, Fraunhofer Institute for Molecular Biology and Applied Ecology, Germany, (5)Ecotoxicogenomics, Fraunhofer Institute for Molecular Biology and Applied Ecology, Schmallenberg, Germany

Global use of antidepressants is steadily increasing, with incomplete removal during wastewater treatment raising concerns about risks to aquatic ecosystems. Antidepressants act on the neuronal system by affecting neurotransmitter levels and have shown to cause behavioural effects in aquatic organisms. While behavioural effects are often attributed directly to altered neurotransmitter levels, there is a knowledge gap regarding whether effects on the visual system also contribute to these effects. This work aims to assess adverse effects of antidepressants on the visual system of early life stage zebrafish and to determine the underlying mechanism. Embryos were exposed to sublethal concentrations of the tricyclic antidepressants amitriptyline (AMI) and nortriptyline (NOR) and the selective serotonin reuptake inhibitor sertraline (SER). Effects to visual function were assessed via the optokinetic response (OKR) assay, cellular structure of the eye via histology, and gene expression via mRNA sequencing and qPCR. The OKR assay, which measures eye movements in reaction to an optical stimulus, showed significant effects for 4.99 and

234 µg/L of AMI and 20.7 µg/L of NOR (measured concentrations), with zebrafish embryos exhibiting 24%, 83% and 60% less eye saccades compared to the solvent control (0.01% DMSO) respectively. Histology revealed a significant increase in retinal pigment epithelium (RPE) thickness for both the 234 µg/L AMI (13.34 µm) and 20.7 µg/L NOR (13.29 µm) groups, compared to the solvent control (11.52 µm). Transcriptomics revealed significant effects to processes including synaptic signalling, neuron morphogenesis, and visual perception. Among the differentially expressed genes associated with visual perception, several genes involved in photoreception (*opn1mw1*, *rho*) and vision (*arr3a*, *pde6c*) were dysregulated. OKR assay with SER showed a significant decrease in eye saccades compared to the control, 34% and 86% respectively, at 100 and 1000 µg/L (nominal). Histology revealed a significant decrease by 17% in RPE thickness for 1000 µg/L SER.

The results provide evidence that antidepressants can induce ocular toxicity in early life stage zebrafish on multiple levels of biological organization. While serotonin is implied to be involved in the mechanism, current experiments with the transgenic zebrafish line *tg(tg:mCherry)* focus on testing the hypothesis that thyroid disruption is the mechanism connecting altered serotonin levels to ocular toxicity. The authors would like to thank the European Commission and the Ministry of University and Research (MUR, Italy), the Federal Ministry of Education and Research (BMBF, Germany), the Research Council of Norway (RCN, Norway) and State Research Agency (AEI, Spain) for funding in the frame of the collaborative international consortium PHARMASEA financed under the 2020 AquaticPollutants Joint call of the AquaticPollutants ERA-NET Cofund (GA N° 869178). This ERA-NET is an integral part of the activities developed by the Water, Oceans and AMR JPIs. The PhD grant of Marwin Jafari is funded by the University of Stavanger (Norway).

2.04.P-Tu175 High-Content Screening in *C. elegans* for Neuronanosafety: Impacts of Silver Nanoparticles on Neurodegeneration

Nivedita Chatterjee¹, Marie Pierron², Laurent Mouchiroud² and Ernesto Alfaro-Moreno¹, (1)International Iberian Nanotechnology Laboratory, Portugal, (2)Nagi Bioscience, Switzerland

With the increasing production and use of nanomaterials, concerns about their neurotoxic potential have risen, prompting investigations into their effects on neuronal health. *Caenorhabditis elegans* (*C. elegans*), with its fully mapped nervous system and conserved genes, serves as an excellent model for studying neurotoxicity due to its genetic tractability and rapid neurobehavioral assessments. As a New Approach Methodology (NAM) tool-kit, it provides a cost-effective, ethical alternative to traditional animal models, enabling high-content screening and nanosafety research. This study evaluates the neurobehavioral and neurodegenerative effects of silver nanoparticles (AgNPs) on *C. elegans* using the high-content screening platform SydLab™ One from Nagi Bioscience. Significant growth inhibition and developmental delays were observed at higher concentrations (0.5 and 5 µg/mL) of AgNPs, with a mean growth (area) rate of 2.56 ± 0.49 µm²/hour for controls, compared to 1.45 ± 0.28 for 0.5 µg/mL. Reproductive assessments showed reduced progeny accumulation, with a mean rate of 2.4 ± 1.75 progeny/hour at 0.5 µg/mL compared to 4.15 ± 1.77 for controls, and no progeny observed at 5 µg/mL until 80 hours, indicating reproductive failure at highest concentrations. Additionally, we investigated the differential neurobehavioral and neurodegenerative effects of AgNPs (0.05 µg/mL) across wild-type and neurodegenerative disease models (Alzheimer's disease model, GMC101 and Parkinson's disease model, NL5901) over 11 days. Survival (% viability) and behavioral endpoints, including mean body bending frequency (Hz) and locomotion speed (mm/sec), indicated increased sensitivity to AgNPs in the Alzheimer's disease model (GMC101). In contrast, the Parkinson's disease model (NL5901) showed no significant differential effect. This increased sensitivity in the Alzheimer's model was supported by glutamatergic neurodegeneration observed in the reporter strain (DA1240). These findings suggest that AgNPs exposure may be associated with amyloid-beta aggregation, potentially amplifying Alzheimer's disease risk. Overall, *C. elegans* proves to be a valuable model for high-content neurobehavioral screening, offering predictive insights into nanoparticle effects on vulnerable populations, making it an ideal tool for NAM in neurotoxicology and nanosafety research. This study was funded by the European Union's H2020 projects, Sinfonia (N.857253), LEARN (N.101057510) and iCare (N.101092971).

2.04.P-Tu176 Integrating Habitat Selection Response with Biochemical and Behavioral Effects in Zebrafish Caused by Glyphosate Under Both Forced and Non-Forced Exposures

Jaine Ames¹, Beatriz Barbosa Moreno², Safa Bejaoui³, Nejla Soudani³, David Salvatierra⁴, Giseli Swertes Rocha⁵, Maria Pilar Gonzalez⁴, Maria Ubeda-Manazanaro⁴, Vania L. Loro¹ and Cristiano V.M. Araujo⁴, (1)Universidade Federal de Santa Maria, Brazil, (2)Universidade Federal de São Paulo, Brazil, (3)University of Tunis El Manar, Tunisia, (4)Institute of Marine Sciences of Andalusia, Spain, (5)Universitat Rovira I Virgili, Spain

Glyphosate is a widely used pesticide in agriculture, with high occurrence in aquatic systems and huge

potential to affect non-target species. Among the problems caused by glyphosate, the behavior modulation of organisms deserves special concern. Behavior results from a reaction to an environmental stimulus, processed through physiological mechanisms. Therefore, any behavioral response relies on a biochemical processing process. Although many studies have demonstrated the harmful effects of glyphosate on aquatic species, the integration of the traditional exposure approach (forced exposure) with scenarios with a chemically heterogeneous environment (non-forced exposure systems) has not been addressed. Therefore, the present study aimed to evaluate the effect of contamination by glyphosate on the habitat selection (avoidance and colonization) behavior of zebrafish (*Danio rerio*) under a non-forced exposure scenario (HeMHAS - Heterogeneous Multi-Habitat Assay System), in which fish were exposed to a glyphosate gradient. Secondly, it aimed to integrate the habitat selection response with biochemical alterations (oxidative damage and enzymatic antioxidant defense) and behavioral changes (swimming pattern, sociability, aggressiveness, and predatory response) measured under a forced exposure approach. The concentrations in all the experiments were: 0, 5, 50, and 100 $\mu\text{g/L}$. In habitat selection tests, zebrafish tended to prefer the least contaminated compartments, either in colonization or avoidance tests. On forced exposure, fish changed their swimming and foraging behaviors, which can represent a serious risk to the species' traits and survival. All the biochemical markers (levels of hydrogen and lipid peroxide, malondialdehyde, protein, ferric reducing antioxidant potential, reduced glutathione, glutathione-S-transferase and peroxidase, metallothionein, catalase, carboxylesterase, superoxide dismutase and acetylcholinesterase and $\text{Na}^+\text{K}^+\text{ATPase}$ activity) were affected by glyphosate in a concentration-dependent relationship. A correspondence was observed between selection habitat and behavior and between markers of oxidative stress, including oxidative damage and activation of antioxidant defense mechanisms. In summary, habitat selection represents a particularly sensitive indicator, closely linked to behavioral and biochemical alterations. These interactions could enrich approaches for assessing environmental risks associated with contaminants.

2.04.P-Tu177 Impacts of the Antidepressant Fluoxetine on Behaviours and Immune Responses in Fish

Gabriel Melhado and Bob Wong, School of Biological Sciences, Monash University, Australia

Pharmaceutical pollution in freshwater ecosystems raises global environmental concerns, particularly regarding its potential impact on the fitness of aquatic wildlife. Among pharmaceuticals, antidepressants pose specific risks due to their effects on neurological mechanisms. One antidepressant of emerging concern is fluoxetine, a selective serotonin reuptake inhibitor (SSRI) commonly prescribed for treating depression and anxiety in humans. Fluoxetine can disrupt behavioural regulation by impacting serotonin reabsorption in the brain. Changes in serotonin levels can lead to disturbances to key behaviours (i.e., locomotion, feeding, and aggression) that are essential for survival. Furthermore, since serotonin plays a vital role in regulating both behavioural and physiological responses, alterations in serotonin levels due to fluoxetine exposure may also be expected to affect immune function. In turn, this can influence the ability of organisms to cope with potential infections and diseases in the wild. Accordingly, this study investigated the effects of 28-day fluoxetine exposure at three environmentally realistic concentrations control (0 ng/L), low fluoxetine (measured average concentration: 4 ng/L), and high fluoxetine (measured average concentration: 637 ng/L) on body condition, behaviours and immune function of rainbowfish (*Melanotaenia fluviatilis*). Body mass and behavioural responses, including distance travelled, average velocity, time freezing, and shelter use, were assessed alongside immune traits, such as total white blood cell (WBC) counts and the neutrophil/lymphocyte (N/L) ratio. While no significant changes were found in body condition or behaviours, fluoxetine exposure decreased WBC counts and increased the N/L ratio. Our findings highlight the potential immunosuppressive effects of fluoxetine on aquatic species, even in the absence of behavioural alterations, suggesting that freshwater pollution caused by pharmaceuticals may have multifaceted impacts on the fitness and survival of aquatic species in contaminated freshwater habitats. This work was supported by the Society of Environmental Toxicology and Chemistry (SETAC) Australasia and the Australian Council of Environmental Deans and Directors (ACEDD) "Peter Teasdale Memorial Award".

2.04.P-Tu178 Neurobehavioural Effects of Bisphenol S on the Early Life Stages in Zebrafish (*Danio rerio*)

A.K.M. Munzurul Hasan, Mahesh Rachamalla, Sravan Kumar Putnala, Md Helal Uddin, Som Niyogi and Douglas P. Chivers, Department of Biology, University of Saskatchewan, Canada

Bisphenol S (BPS) is a widely used synthetic compound known as an endocrine-disrupting chemical (EDC). It is commonly found in epoxy resins and polycarbonate plastics, materials frequently used for food storage containers and baby bottles. The ability of BPS to bind predominantly to estrogen receptors raises significant concern, as it can interfere with different neurological functions leading to

neurobehavioural deficits. Despite extensive research documenting various adverse effects of BPS in adult fish, its neurobehavioural effects, especially in early life stages of fish, remain poorly understood. In the present study, zebrafish embryos (4-hour post fertilization, hpf) were exposed to environmentally relevant concentration of BPS (vehicle control, 0 and 30 µg/l) until 120 hpf. Subsequently, behavioural effects of BPS were evaluated by examining various behavioural responses including thigmotaxis (120 hpf), reflexive movement (120 hpf), social preference (21 dpf (day post fertilization), and object recognition (26 dpf). These results indicated that exposure to BPS impaired thigmotaxis, reflexive movement, social and object recognition behaviours in zebrafish larvae. In addition, reactive oxygen species (ROS) production and apoptosis were evaluated at 120 hpf zebrafish larvae, which were found to be elevated following exposure to BPS. Moreover, gene expression analysis at 120 hpf indicated that BPS exposure resulted in the dysregulation of genes involved in serotonergic (*slc6a4a*, *slc6a4b*, *tph2*, *htr1aa*, *htr1ab*, *htr1b*, *htr1d*, *htr2aa*, *htr2b*) and cholinergic (*ache*, *chata*, *creb1a*) neurotransmitter pathways, apoptosis pathway (caspase-3, p-53), oxidative stress response (*gpx*, *mn-sod*), and neuroinflammation (*il-1?*, *il-6*). These findings suggest that BPS induces oxidative stress and apoptosis, which likely leads to disruption of neural development and signaling pathways involved in regulating behavioural responses. Overall, our study highlights that developmental exposure to BPS can induce marked behavioural deficits in larval zebrafish, and provides new insights into the neurotoxic mechanisms underlying its behavioural effects.

2.04.P-Tu179 From Brain to Sperm: how Psychoactive Drugs Can Affect Fish Reproduction

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There is a growing concern about the occurrence of psychoactive drugs in the aquatic environment. Recent studies indicate adverse effects of psychoactive drugs on aquatic life at environmentally relevant concentrations. Several studies confirm behavioural changes in fish and crayfish exposed to psychoactive drugs. Despite this, very little is known about the mechanisms of those changes. Psychoactive drugs have a specific action on one or more neurotransmitters or neuroreceptors, which are present in neuron cells in both humans and fish. Individual targets are well conserved, suggesting that psychoactive drugs in fish act through similar mechanisms as in humans. Neuronal signaling is important not only for brain function but also for sperm function. It is regulated by signals, several of which correspond to neurotransmitters that activate the transduction signaling implicated in the molecular control of sperm physiology. Mammalian spermatozoa express receptors for many neurotransmitters and neuromodulators. Information on the presence of neurotransmitters and their receptors in fish sperm and gonads is limited. If neurotransmitters may be involved in sperm physiology, then constant exposure of fish to psychoactive compounds, which are ubiquitous in the aquatic environment, may lead not only to behavioural changes but also affect sperm functioning and, consequently, reproduction. The specific scope of the study was to do a comprehensive analysis of the common neurotransmitters (monoamines, trace amines, amino acids, and others, as well as some of their precursors and metabolites) in the brain, gonads and sperm of the European perch (*Perca fluviatilis*), and then check if they will be altered by the exposure to psychoactive drugs. Among 31 analyzed neurotransmitters (including some of their precursors and metabolites), 22 were determined in the brain, 19 in the gonad, and 15 in the sperm. Psychoactive drugs were bioaccumulated in all three tissues. Brain and gonads had similar levels, while sperm had slightly lower concentrations. There was a significant decrease in glutamate concentration in sperm after 7 and 23 days of exposure to methamphetamine, while there was slight increase in the brain after 23 days. This may have potential effects on fish sperm function that, in turn, may influence fish reproduction. The authors thank the Czech Science Foundation [project No. 22-03754S], The South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses (CENAKVA, ID 90238, MEYS CR)

2.04.P-Tu180 Exploring the Molecular Basis of Transient versus Persistent Behavioral Alterations Using a Multi-Omics Approach in the Zebrafish Model

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Chemical-induced alterations in fish behavior can occur through specific disruptions of the nervous system or nonspecific physiological disturbances. Zebrafish (*Danio rerio*) larvae serve as a model for assessing neurotoxicity, particularly in locomotion studies. Our research investigates the transient and reversible nature of behavioral changes induced by the neuroactive pharmaceuticals buspirone and fentanyl, analyzing their effects through an exposure/depuration experimental setup. Utilizing transcriptomics (RNAseq), global proteomics and targeted metabolomics, we aimed to elucidate molecular

responses associated with these drugs during a 9-day assessment period. Larvae were exposed to either buspirone or fentanyl continuously for 6 days and subsequently placed in chemical-free medium for 3 days. Behavioral assessments revealed reduced locomotor activity induced by both chemicals, with recovery profiles indicating distinct patterns: larvae depurated from buspirone exhibited restored locomotion in dark phases, while those exposed to fentanyl showed recovery only in light phases. Our neurochemical analysis demonstrated rapid recovery of serotonin levels in buspirone-exposed larvae, while adrenaline levels remained consistently low across both treatments. Notably, noradrenaline exhibited quicker recovery in buspirone-exposed larvae compared to gradual recovery in fentanyl-exposed counterparts. Proteomic analysis revealed common alterations in metabolic pathways, oxidative phosphorylation, motor proteins, and cardiac muscle contraction, alongside specific alterations such as impacts on the apelin signaling pathway with buspirone and necroptosis pathway modulation with fentanyl. Evaluation of the RNAseq data and its integration with proteomics and metabolomics results is currently ongoing and expected to further increase the depth of molecular insights gained from this work. Our findings enhance the understanding of neuroactive compounds effects on zebrafish locomotion and facilitate insights into the underlying molecular mechanisms. The identified molecular markers can inform assessments of lethal, sublethal, and behavioral effects during early developmental stages, contributing to a comprehensive understanding of neurotoxic impacts on fish and aiding future ecotoxicological interpretations of behavioral effects.

2.04.P-Tu181 Early Life Stress Disrupts Later Life Coping Behaviours in Zebrafish

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Animals experience stressful unpredictable conditions throughout their life cycle, including critical developmental periods. Stress at early life stages may affect brain function, leading to persistent behavioural disruption and potentially the expression of personality traits. However, the currently available data is limited, making it unclear if and how early-life stress impacts personality development, animal fitness, and the ability to cope with other environmental stressors, such as contaminants. This study examined the consequences of abiotic stress during different early life stages of zebrafish development on later coping behaviours to identify the most sensitive developmental stage(s). Animals were chased with a net for 20 sec, subjected to high aeration, low temperature ($8.0 \pm 1^\circ\text{C}$) for 30 sec and air exposure for 5 sec after recovering normal activity from low-temperature exposure. Stress was applied at three specific developmental stages according to organisms' tolerance and temporal patterns of cortisol synthesis: at 96 hours post fertilisation (hpf), 7 days post fertilisation (dpf) and 15 dpf. They were then reared under standard zebrafish housing conditions until the juvenile stage (45 dpf). The control group was not exposed to any stressors and was kept under the same conditions as the other experimental groups. At 45 dpf a battery of behavioural phenotyping trials was carefully selected to characterise bold and shy behavioural profiles among experimental groups to assess the modulatory role of stress on personality expression. Five independent assays were conducted, each in triplicate, with three replicates per condition. Overall, the results show that stress during early development plays a key modulatory role in later behavioural phenotype expression. The differences between shy and bold phenotypes observed in the control group were lost at 45 dpf in fish exposed to early-life stress for all the tested behavioural endpoints (total distance swum, inactivity time, and aggressiveness) and independent of the developmental stage exposed to stress. Bold individuals appeared to be more susceptible to early stress, especially those exposed to stress at 4 and 7 dpf, suggesting reduced phenotypic plasticity and adaptive flexibility. These outcomes suggest that early-life stress may compromise animals' fitness potentially affecting their sensibility and ability to cope and adapt to contaminants. Thanks are due to the financial support to CESAM by FCT - Foundation for Science and Technology (UIDB/50017/2020 + UIDP/50017/2020+LA/P/0094/2020) and to FCT/MCTES for the financial support to the project NanoPlanet (DOI10.54499/2022.02340.PTDC). FCT supported Carla Melo through a PhD Grant (2021.04580.BD).

2.04.P-Tu182 Assessing Neurodevelopmental Impacts of a Novel Bio-Hybrid Fuel for Combustion Engines in Zebrafish

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A crucial step towards a carbon-neutral future is transitioning the transportation sector to renewable energy sources. One promising approach is the development of bio-hybrid fuels, which utilizes bio-based feedstocks, carbon dioxide, and green hydrogen produced from renewable energy. While bio-hybrid fuels offer significant advantages from an engineering and environmental perspective, including increased

engine efficiency and reduced greenhouse gas emissions, it is imperative to assess their human and environmental impacts early during their development. Specifically, certain compounds within these fuels may pose neurotoxic risks during production, handling, or accidental release. Using a multi-level neurotoxicological approach, we systematically assessed whether and to what extent the bio-hybrid fuel blend candidate KEAA (ketone-ester-alcohol-alkane) induces neurodevelopmental changes in zebrafish embryos up to 120 hours post fertilization (hpf). We examined sublethal effects on coiling activity (24 hpf), touch-evoked response (72 hpf), and swimming behavior (120 hpf) in the Light/Dark-Transition Test. These behavioral analyses were complemented by morphological and enzymatic analyses, including measurements of body length, eye size, yolk sac size, swim bladder inflation, as well as acetylcholinesterase (AChE) inhibition. Results show that KEAA exposure exerts a clear, concentration-dependent effect on zebrafish larval morphology. At an incubation temperature of 26 °C, concentrations above 113 mg/L decrease body length and eye size, while yolk sac depletion is significantly slower at 120 hpf. AChE inhibition is 50 % at a concentration of 316 mg/L at 26 °C but show a biphasic concentration-response at an incubation temperature of 28 °C. This suggests that neurotoxic effects at exposure concentrations near the EC10 are temperature-sensitive. These findings provide essential early-stage data on the neurotoxic potential of bio-hybrid fuels like KEAA, informing safer fuel design to minimize human and environmental risks. This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy Cluster of Excellence 2186 The Fuel Science Center ID: 390919832.

2.04.P-Tu183 Psychoactive Pollutant Alters Movement Dynamics of Fish in a Natural Lake System

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Pharmaceutical pollution poses an increasing threat to global wildlife. Psychoactive pharmaceutical pollutants (e.g. antidepressants, anxiolytics) are a distinctive concern due to their ability to act on neural pathways that alter fitness-related behavioural traits. However, despite increasing research efforts, very little is still known about how these drugs influence the behaviour and survival of species in the wild. Here, we capitalise on the development of novel slow-release pharmaceutical implants and acoustic telemetry tracking tools to investigate how the commonly detected psychoactive pollutant temazepam (an anxiolytic) influences the movement dynamics of brown trout (*Salmo trutta*) in a natural lake system. Briefly, 90 brown trout were administered with a slow-release implant containing either no pharmaceuticals (i.e. control [$\mu\text{g/g}$]) or temazepam [$50 \mu\text{g/g}$] that mimics tissue concentrations that fish commonly experience in the wild. Fish were then released into Lake Orsa in central Sweden, where their movement was tracked for ~10 months. We found that temazepam altered movement dynamics and decreased the migration success of brown trout in the wild most likely due to temazepam-exposed fish suffering increased predation compared to unexposed conspecifics. These findings underscore the potential for pharmaceutical pollution to alter key fitness-related behavioural traits under natural conditions. Funding was provided by the Swedish Research Council Formas (2020-02293 to M.G.B.; 2020-00981 to E.S.M.; 2020-01052 to D.C.; 2022-00503 to M.M.; 2018-00828 to T.B.), the Kempe Foundations (SMK-1954, SMK21-0069, and JCSMK23-0078 to M.G.B.), the Marie-Claire Cronstedt Foundation (to M.G.B.), the ÅForsk Foundation (20-51 to M.G.B.), the Orsa Fisheries Conservation Area Association (to D.P.), the Oscar and Lili Lamm Memorial Foundation (FO2021-0016 to M.M.), and the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska Curie grant agreement (101061889 to M.M.).

2.04.P-Tu184 Behavioral and Reproductive Effects of the Antidepressant Fluoxetine Associated With Traditional and Compostable Microplastics

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The aquatic environment faces increasing challenges due to the introduction of diverse pollutants, such as microplastics and pharmaceutical residues like antidepressants, which pose significant threats to aquatic life. Fluoxetine (FLU), a widely prescribed antidepressant, stands out among these pollutants due to its prevalence and hydrophobic nature, which may lead to its adsorption onto matrices such as sediments and plastic fragments. Microplastics (MPs), particularly those smaller than 5 mm, ubiquitously permeate aquatic environments, acting as vectors for pollutants and posing direct or indirect threats to aquatic organisms. Efforts to mitigate plastic pollution have led to the development of biodegradable and

compostable polymers aimed at reducing environmental persistence. However, these alternative materials may also generate microplastics (CMPs) capable of transporting pollutants. The interaction of CMPs with organisms remains understudied compared to traditional MPs. Recent studies have demonstrated the adsorption of FLU onto various aged MPs, raising concerns about the potential ecological impacts of these interactions. Ecotoxicological assessments, using model organisms such as *Daphnia magna*, are crucial to comprehensively understanding the impacts of FLU-contaminated CMPs and traditional MPs on aquatic organisms. Sub-lethal effects, particularly on behavior and reproduction, require careful evaluation in those assessments. Therefore, the aim of the current study was to evaluate the effects of traditional and compostable microplastics associated with FLU on behavior and reproduction of *D. magna*. The behavioral evaluations include unforced exposure methods, such as the avoidance and colonization tests using the Heterogeneous Multi-Habitat Assay System (HeMHAS), designed to simulate real-world scenarios and assess ecological risks. The avoidance and colonization evaluation were carried out using a multi-habitat scenario, with the control at the center and contaminant variables arranged around it, aiming to detect repellence and attraction to the MP, CMPs or FLU. Reproduction after 21 days exposure, showed that the mixture of traditional MPs with the highest concentration of FLU had an influence on the number of offspring compared to other treatments, demonstrating that the interaction between these two pollutants is concerning and requires further study to understand its potential environmental impacts.

2.04.P-Tu185 Alterations in Behaviour After Exposure to Leachate from SARS CoV-2 Colloidal Gold Test Strips in *Danio rerio* (Zebrafish)

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Nanomaterials are used to improve various industries due to their unique properties, and this increase in use results in the increased prevalence of these materials in the environment. Since nanogold is thought to be safe and biocompatible, it has gained interest for possible applications in the medical industry. Thus, conducting toxicological assessments is important to understand the health risks that may be caused by nanogold. There are conflicting reports on the cytotoxicity of nanogold, with some claiming it is safe and compatible while others claim it is capable of bioaccumulation in various tissues and capable of inducing mitochondrial damage. Zebrafish are a useful model due to their genetic similarities with humans and other mammals and are frequently used to assess behavioural changes induced by different substances. Zebrafish are highly social and can thus be used to assess memory by using social interactions as a reward. To confirm whether SARS CoV-2 colloidal gold tests kits are capable of leaching nanogold into the environment scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS) and UV-vis spectroscopy were used, and attenuated Total Reflectance-Fourier Transform Infrared spectroscopy (FTIR-ATR) was used to test for other chemicals present on the test strips. Using ViewPoint digital tracking software, a novel tank diving test was then conducted to assess the baseline behaviour of the experimental fish in novel environments, after which they were trained in two sessions to only be familiarised with two zones of a 3D-printed T-maze by using conspecifics as a visual reward. Lastly, the behaviour of the fish following exposure to leachate, alarm substance from donor fish, and a combination of both was quantified by removing the conspecifics and further quantifying behaviour. Prior to exposure, the control group displayed risk-taking behaviour which remained consistent after removing the conspecifics from the maze. The fish exposed to leachate, alarm substance, and a combination of both displayed increased anxiety-like behaviour compared to the control group and did not explore the unfamiliar zone of the maze. There were statistically significant differences between the overall time spent moving or distance travelled in specific zones within the maze compared between exposure groups. This study highlights that further research is required in the safe development of commercially available nano-based products.

2.04.P-Tu186 In Silico Pre-Screening to Identify Neurotoxic Substances Which Are Considered Out of Applicability Domain for The Fish Embryo Acute Toxicity (FET) Test

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The classification hazardous to the aquatic environment under CLP regulation relies on data from fish, and generation of such data raises animal welfare concerns. The Fish Embryo Acute Toxicity (FET) test is a potential alternative to acute fish toxicity study, but neurotoxic substances are considered out of the applicability domain of the method. The aim of the project was to evaluate how well (Q)SAR models can predict neurotoxicity, and if these (Q)SAR models could be a feasible screening tool to identify neurotoxic substances and thus out of the applicability domain when considering FET test as an alternative to acute fish toxicity study. The ability of (Q)SARs to predict neurotoxicity and identify neurotoxic substances was

evaluated using 113 neurotoxic substances (harmonised classification as STOT RE 1/2 or STOT SE 1/2/3 with the nervous system as target organ or because they induce narcotic effects). These substances fall within the scope of REACH or are active substances in biocidal or plant protection products. The following (Q)SAR models, predicting neurotoxicity and inhibition of acetylcholinesterase, were used: Derek Nexus 2.6, ASTER (Assessment Tools for the Evaluation of Risk), ProTox 3.0, and Linear discriminant analysis model. Models were applied only to mono-constituent organic substances (89/113). Publicly available SMILES were mainly derived from the US EPA CompTox Chemicals Dashboard. Only 36% of neurotoxic substances (32/89) were identified as potential neurotoxicants (positive prediction) by one or more (Q)SAR models. The positive predictivity of individual models ranged from 4.5% (4/89) to 22.5% (20/89) with only four substances predicted as positive by more than one model. These results indicate that the predictive capacity of each model alone is low, but could be slightly improved by combining results from all four models. The ability to identify the neurotoxicants by the models was influenced by the substance type (i.e. ASTER and LDA models provided positive predictions only for substances registered for biocidal or plant protection products). As a conclusion, the selected (Q)SAR models were not able to identify majority of the neurotoxicants included in this study, and therefore would not serve as a reliable screening tool for substances that are outside of the applicability domain for the FET. In general, the findings indicate a need for better (Q)SAR models for improved identification of potential neurotoxic substances.

2.04.P-Tu187 Neurobehavioral and Neurochemical Effects of Nano-Polypropylene Accumulation in Zebrafish (*Danio rerio*)

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Plastic pollution, particularly nanoplastics (NPs), is a significant environmental contaminant that poses potential toxicological risks to organisms and ecosystems. Although extensive research is currently underway to study the toxicity of NPs, our understanding remains limited, primarily because of the constraints of standardized toxicity studies using polymers of specific sizes and types. To address this gap, we conducted toxicity experiments using directly synthesized polypropylene nanoparticles (PP-NPs) in zebrafish (*Danio rerio*). The presence of PP-NPs in the zebrafish brain was confirmed using pyrolysis gas chromatography-mass spectrometry (Py-GC/MS) and bio-transmission electron microscopy (bio-TEM). The accumulation of PP-NPs in the brain of *D. rerio* leads to neurotoxicity, reducing motility and aggressiveness. Changes in neurotransmitter levels and neural activity associated with behavior further supported these findings. This study suggests that environmental plastic pollutants may accumulate in the brain and cause neurotoxicity in organisms, emphasizing the need for appropriate management of these substances.

2.04.P-Tu188 Flazasulfuron Based-Herbicide and Glyphosate Effects on Zebrafish (*Danio rerio*) Larvae Behavior

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Flazasulfuron (FLA) and glyphosate (GLY) are two non-selective herbicides commonly applied in agriculture. Consequently, their overuse and cumulative practices lead to environmental contamination of aquatic ecosystems. However, few studies dealing with FLA's ecotoxicity to non-target biota are available, and for GLY there is a lot of divergent data regarding its toxic effects. The present study aimed to evaluate the effects of a flazasulfuron-based herbicide and glyphosate on zebrafish (*Danio rerio*) larvae behavior. For this, zebrafish larvae at 7 days old were exposed to 6 treatments: control (E3 medium), dimethyl sulfoxide (DMSO, 0.01%), commercial glyphosate-based herbicide (30 µg/L), commercial flazasulfuron-based herbicide (30 µg/L), and to the respective active ingredients flazasulfuron (30 µg/L) and iodosulfuron-methyl-sodium (10 µg/L). Based on the profile simulations using the Exposure Pattern Analysis Tool (EPAT), an exposure of 24h to the treatments, followed by a recovery period of 10 days, and a new exposure of 36h, was defined. Four replicates were established, with 20 larvae per group. Larvae were maintained at 28 ± 0.5°C and 14:10 h (light: dark) photoperiod. Mortality was assessed daily, and after each exposure period (24 and 36 h), 8-10 animals were sampled from each group/replicate for behavioral assessment. The behavior of larvae was monitored using a video-tracking system (TheRealFishTracker), and the following parameters were evaluated: locomotor performance (e.g. mean speed, total distance moved, mean absolute turn angle), social (average inter-individual distance IID; and the nearest neighbor distance - NND) and avoidance/anxiety behaviors. Considering the results, the

survival and development of larvae were not significantly affected by the treatments, despite glyphosate presenting a higher mortality rate than the remaining groups. After 24h of exposure, neither the locomotor nor the social behavior were affected by the herbicides and active ingredients ($p>0.05$). However, after the second exposure period of 36h, it was observed a higher absolute turn angle in larvae of the flazasulfuron-based herbicide exposed group ($p<0.01$). The absolute turn angle is considered a sensitive parameter to evaluate motor coordination, therefore changes in this parameter could indicate a disorganized locomotor pattern. Our study evaluated the ecotoxicity of FLA and GLY on larvae behavior, helping to further advance herbicide risk assessment. This work is funded by the Project Vine&Wine Portugal Driving Sustainable Growth Through Smart Innovation, Ref. no. C644866286- 00000011, co-financed within the scope of Mobilization Agendas for Business Innovation, under Reg. (EU) 2021/241, in the Recovery and Resilience Plan (PRR) of Portugal, and by National Funds by FCT - Portuguese Foundation for Science and Technology, under the project UIDB/04033/2020.

2.04.P-Tu189 Innovative AI-Driven Bioluminescence Paper Biosensing: Advancing Rapid Toxicity Testing and Next-Generation Risk Assessment through Integrated Analytical and Computational Approaches

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Water monitoring is essential for ecosystems and human well-being and plays a crucial role in sustaining life and health. The ISO 11348 method, based on either freshly prepared or freeze-dried bioluminescence marine Gram-negative bacteria *Aliivibrio fischeri*, is widely used to monitor water quality. *A. fischeri* emit photons of visible light as a byproduct of their metabolism, but since their viability is affected by toxic agents, the decrease in bioluminescence can be used to assess the toxicity of water and environmental samples. However, this method requires benchtop instrumentation, facilities for bacterial cultures and skilled personnel, with high cost and is time-consuming. To turn this method into a portable, low-cost, and user-friendly toxicity biosensor, we developed a toxicity sensing paper composed of immobilized bacteria on a paper-based platform that exploits a smartphone camera as a light detector. The paper biosensor included 6 wells for the calibration curve and a central well for the sample. In addition, a customized android application, named Scentinel App, was developed to analyze the image of the toxicity sensing paper and to convert the decrease in light intensity into quantitative information about sample toxicity. The analytical performance of the biosensor was evaluated with different water contaminants such as NaClO, microcystin-LR, 3-5 dichlorophenol, and lead nitrate, and with real water samples, showing promising results. The app combined with the paper sensor was also evaluated with different smartphone models, showing promising results both in terms of analytical performance and ease-of-use with potential applicability for use by the general population. The authors thank for funding the European Union's Horizon Europe project FARMWISE under GA No. 101135533

2.04.P-Tu190 An Effect of Color? Impact Of Food Dyes and Micro(Nano)Plastics on Fish

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Humans are increasingly exposed to an array of potential materials that may compromise their health. Among the substances that are raising health concerns are artificial food dyes, that have seen an increased consumption over the last decades. Despite regulatory agencies efforts to assure their safety, the current available data concerning their effects is limited and often contradictory. Furthermore, these substances may interact with other materials involved in food production and packaging. Thus, this study focused on the acute effects of artificial food dyes on early life stages of *Danio rerio*. Its effects were also studied in combined exposure with micro(nano)plastics, products that are also raising concern regarding their potential effects on human health. Larvae were exposed for 96 hours to a range of concentrations of food dyes and mortality, morphological abnormalities and behaviour endpoints (e.g., swimming performance) were assessed. The obtained results provide relevant information for a more robust risk assessment of food dyes and highlight the need of more studies on the subject. Thanks are due to Portuguese Fundação para a Ciência e a Tecnologia (FCT)/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020+LA/P/0094/2020) and the project Nano Planet (DOI: 10.54499/2022.02340.PTDC) through national funds.

2.04.P-Tu191 Fish on Zoloft: Physiological and Behavioural Effects of Sertraline on *Danio rerio* Early Life Stages

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Antidepressants, widely consumed pharmaceuticals, often enter the environment through patient excretion

and improper disposal. Wastewater treatment plants are insufficient at fully removing these drugs and their active metabolites. Designed for biological effects at low concentrations, antidepressants may pose hazards to non-target organisms. Sertraline, a selective serotonin-reuptake inhibitor, is the most consumed drug in its class and has been detected in various aquatic systems, where it may exert biological effects. This study aimed to assess lethal and sublethal effects of sertraline on embryos and larvae of *Danio rerio* and assess the influence of stress coping styles (bold-shy continuum). *Danio rerio* embryos and larvae were exposed to five concentrations of sertraline (0.21–5 mg L⁻¹) plus a negative control (E3 medium) for 144 and 96h, respectively. Lethal and sublethal effects, including mortality and malformations, heart rate (48h) and behaviour, were assessed at the end of each exposure period. For the personality assay, embryos exposed to 0, 0.01 and 0.1 µg L⁻¹ sertraline until 8dpf, were classified in a bold-shy axis using a group risk-taking test, and fitness-related behaviours were analysed using the a tracking system. Mortality reached 100% at the highest tested concentrations (i.e., 5 and 2.27 mg L⁻¹), while at 1.03 mg L⁻¹ a 45% malformation rate was observed, excluding this group from behavioural analysis. Under dark periods, embryos exposed for 144h exhibited, compared to control, a less anxious behaviour at 0.21 mg L⁻¹, with decreased swimming distance, time and distance spent in the outer area and increased inactivity time, while at 0.57 mg L⁻¹ decreased swimming distance and increased inactivity time were observed. During light period, angles of class 1 and 8 percentages decreased at 0.57 mg L⁻¹. In a stressful context (light-dark transition), exposed organisms showed reduced swimming distance, time spent in the outer area, angles of class 4 and 5, while angles of class 1 and 8 and inactive time increased at 0.51 mg L⁻¹ compared to control. For the personality assay, bold larvae exposed to 0.1 µg L⁻¹ exhibited thigmotaxis similar to shy larvae, while control and 0.01 µg L⁻¹ kept significant differences between personality type. These findings suggest sertraline induces significant behavioural changes at non-lethal concentrations, with environmentally relevant levels potentially modulating behavioural traits in stressful contexts.

2.04.P-Tu192 BMAA-Induced Neurotoxicity: Unveiling the Behavioural and Developmental Impacts on Larval Zebrafish

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Rising global temperatures and increasing eutrophication have amplified the prevalence of harmful algal blooms (HABs), posing significant ecological challenges worldwide. Among the toxins produced during HABs, L-N-Methylamino-L-alanine (BMAA), a neurotoxic amino acid synthesized by cyanobacteria, diatoms, and dinoflagellates, has emerged as a critical environmental concern. Investigation of BMAA neurotoxicity to aquatic species is still in its early stages and the molecular mechanism by which BMAA induces its toxicity during the early development of zebrafish remains poorly understood. In this study, zebrafish embryos (2 hours post-fertilization, hpf) were exposed to environmentally relevant concentrations of BMAA (0, 10, 20, and 40 µg/L) until 120 hpf to elucidate its developmental and neurotoxic effects. No significant differences were observed in mortality and deformity rates among treatment groups; however, concentrations of 20 and 40 µg/L impaired thigmotactic and reflexive behaviours in the larval zebrafish. Exposure to BMAA at concentrations of 20 and 40 µg/L led to increased levels of apoptosis and reactive oxygen species (ROS), implicating oxidative stress-induced cell death as a key pathway in BMAA neurotoxicity. This study provides important insights into the neurotoxic effects of BMAA on zebrafish during early development. By examining how BMAA influences zebrafish development and cognition at early stages, our findings contribute to a broader understanding of the ecological impacts of neurotoxic microalgae on aquatic biodiversity, highlighting the need for further research on the long-term consequences of such exposures. Jinnath Rehana Ritu was supported by the Dean's Doctoral Scholarship of the University of Saskatchewan. This work is supported by a discovery grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to Douglas P. Chivers and Maud Ferrari.

2.04.P-Tu193 Early Stage Behavior and Sleeping Pattern Disorder in Zebrafish Larvae After DEP and BBzP Exposure

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The zebrafish, a diurnal vertebrate, is commonly used in circadian rhythm studies due to its genetic and neurological similarities to humans. Circadian rhythms, which regulate sleep, hormones, behavior, and physiological responses to environmental changes, can be disrupted by various environmental factors. Phthalic acid esters (PAEs) are pervasive endocrine disruptors that individuals are frequently exposed to in daily life. However, the impact of PAEs on circadian rhythms during early development remains poorly understood. This study aimed to investigate the effects of exposure to diethyl phthalate (DEP) and butyl

benzyl phthalate (BBzP) on the behavior and circadian rhythms of developing zebrafish larvae using a series of layered assays. Zebrafish larvae were exposed to the two PAEs from less than 2 hour post-fertilization (hpf) until 96 hpf. The results demonstrated a concentration-dependent reduction in tail coiling (TC), touch-evoked response (TER), and locomotor activity, alongside an increase in sleep time and alterations in sleep bouts and sleep latency during both 24-hour and Light1/Dark/Light2 (7/10/7-hour) periods. Additionally, exposure to BBzP led to increased acetylcholinesterase (AChE) and dopamine (DA) levels, and a decrease in 5-hydroxytryptamine (5-HT) levels. Gene expression analysis revealed that DEP and BBzP exposure increased the expression of circadian rhythm and light-response-related genes. In conclusion, exposure to these PAEs disrupts the circadian rhythm of zebrafish larvae, providing novel insights into the developmental impact of these common environmental contaminants. Further research is needed to understand the broader implications of these findings for human health and environmental safety. This study was supported by the Korea Institute of Toxicology (KIT) Research Program (no. 2710008763, KK-2401-01) and Korea Environment Industry & Technology Institute (KEITI) through Technology Development Project for Safety Management of Household Chemical Products Program, funded by Korea Ministry of Environment (MOE) (2022002980005,1485019201)

2.04.P-Tu194 Neurotoxic Effects of Dibutyl Phthalate and its Metabolite in Zebrafish Larvae Model
Suyeon Lee¹, Sangwoo Lee¹, Kojo Eghan² and Woo-Keun Kim¹, (1)korea institute of toxicology, Korea, Republic of, (2)korea institute of toxicology, Ghana

Although dibutyl phthalate (DBP), commonly used as a plasticizer, raises significant neurotoxicity concerns, the effects of the metabolite mono-n-butyl phthalate (MBP) are not yet clear. This study investigates DBP and MBP's developmental and neurotoxic effects on zebrafish (*Danio rerio*) larvae. Wild-type and transgenic (tg(elavl3) and tg(mbp) zebrafish were exposed to DBP and MBP from 4 hpf to 120 hpf. Developmental toxicity was assessed using a variety of parameters, including survival, hatching rate, eye size, deformity, tail coiling, touch-evoked response, and locomotive activity. Neurotoxicity and oxidative stress markers and their associated gene transcription were evaluated by ELISA and qPCR. Fluorescence imaging revealed neurogenesis and demyelination. Studies have shown that both DBP and MBP caused significant changes in touch-evoked response at 72 hpf. Behavioral analysis revealed that DBP exposure affects various aspects, e.g., travel distance, velocity, and turning angle. Gene transcriptional analysis indicated significant effects on neurodevelopmental genes (sox2, manf, gfap) and oxidative stress genes (gst1, gr), providing further insight into the molecular mechanisms of DBP and MBP. Specifically, MBP showed greater influence on molecular markers for neurodevelopment compared to DBP. These findings improve understanding of the neurotoxic potential of DBP and MBP in aquatic species, thus highlighting the importance of considering metabolites in toxicity and risk assessment. This work was supported by the Korea Environmental Industry & Technology Institute (KEITI) through the Core Technology Development Project for Environmental Diseases Prevention and Management [grant number 2480000072 (RS-2021-KE001705)].

2.04.P-Tu195 Ecotoxicological Effects of Imidacloprid: Study of Neurotoxic Impacts on Zebrafish Embryos and *Daphnia Magna*

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Imidacloprid, a widely used neonicotinoid insecticide, has been identified as a contaminant of emerging concern under the Water Framework Directive due to its persistence and potential ecological risks. However, there are still a limited number of studies investigating the ecotoxicological effects of imidacloprid on aquatic ecosystems, leaving critical gaps in understanding its impact on aquatic species, food webs, and overall ecosystem health. The study assesses the environmental impact by detecting acute and neurotoxic effects on fish *Danio rerio* and a crustacean *Daphnia magna*. Acute toxicity was evaluated with the Fish Embryo Toxicity (FET) test (OECD 236, 2013) on zebrafish and Immobilisation test (OECD 202, 2004) on daphnia. EC50 was calculated and sublethal effects such as morphological spine deformation, depigmentation and lack of balance were registered. Neurotoxic effects were evaluated with two internal laboratory protocols: the Coiling Activity Tail (CAT) test, which measures spontaneous tail movements in zebrafish embryos, and a Behaviour Locomotor Activity (BLA) test on daphnia, which describes the effect on velocity, distance and social interaction. The EC50 obtained is in line with the values used for deriving environmental quality standard (EQS) for inland surface water for imidacloprid in the context of the WFD common implementation strategy. The results indicate that imidacloprid exhibits both acute toxicity and neurobehavioral impacts, even at concentrations analogous to those found in aquatic environments. These findings highlight the importance of regulating imidacloprid to mitigate its risks to aquatic ecosystems. Moreover there are still few neuro-ecotoxicological studies on imidacloprid in

literature, and this study can contribute to the implementation of knowledge of potential effects related to environmental risk.

2.05 Lab and Field Collected Invertebrates and in Situ Studies in Ecotoxicology: Challenges and Opportunities

2.05.T-01 Evaluating the Sensitivity of Diverse Freshwater Macroinvertebrates to Chemicals with Different Toxic Modes of Action

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Freshwater macroinvertebrates are increasingly threatened by chemical pollution, with one-third of freshwater insect species at risk of extinction. Predicting chemical impacts on these communities is challenging due to wide variation in species sensitivity. Current ecotoxicological assessments often focus on model organisms like *Daphnia magna*, which do not adequately represent the diversity and sensitivity of broader freshwater ecosystems. Sensitivity to chemicals is influenced by their toxic mode of action (TMoA). Chemicals with broad TMoAs, such as metals, exhibit low variability in species sensitivity, whereas those with specific TMoAs, such as insecticides, produce highly variable responses across species, complicating risk assessments. This study investigates whether species sensitivity to chemicals within a given TMoA follows consistent patterns, enabling predictive modelling for untested chemicals. We tested the acute toxicity of 12 chemicals across four TMoA categories: triazoles, polycyclic aromatic hydrocarbons (PAHs), synthetic pyrethroids, and strobilurins. Toxicity tests were performed on nine UK-relevant freshwater macroinvertebrate species (arthropod and non-arthropod), both field collected and laboratory cultured, representing diverse taxonomic and functional groups. EC₅₀ values were calculated from immobilization data collected over 48–96 hours under controlled conditions. Then, rank order correlations were performed to assess the consistency of species sensitivity across chemicals within and between TMoA groups. Results showed substantial variation in species sensitivity depending on TMoA. Snails were highly sensitive to fungicides but less sensitive to synthetic pyrethroids, while water hoglice were most sensitive to PAHs. We found that the rank order of species sensitivity is more similar within chemicals sharing the same TMoA compared to those with different TMoAs. For example, comparisons between deltamethrin and permethrin showed a high positive correlation (Kendall Tau = 0.852), whereas, comparisons between permethrin and azoxystrobin (different TMoA) showed no/weak negative correlation (Kendall Tau = -0.384). These results confirm that species sensitivity follows predictable patterns within chemicals sharing the same TMoA. This consistent pattern of toxicity allows for more predictable ecotoxicological assessments, reducing the need for extensive testing of new chemicals that are structurally similar. NERC 'Assessing and Managing the Impacts of Mixtures of Chemicals on UK Freshwater Biodiversity in a Changing World' (NE/X015831/1)

2.05.T-02 How to Use Experimental Data and Modelling of the Wild-Caught Non-Standard EPT Taxon *Cloeon dipterum* for Environmental Risk Assessments of Pesticides

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In the European Union (EU), non-standard test species are often used to refine the aquatic risk assessments (RA) of pesticides. Representatives of EPT taxa (Ephemeroptera, Plecoptera and Trichoptera) are of particular interest as they are classed as sensitive and vulnerable to pesticides. The mayfly *Cloeon dipterum* is an abundant species across the UK and Europe, residing in ponds and river margins as larvae. Despite being considered a good representative of EPT taxa in RA (mainly acute studies), there is little published information on the reproducible laboratory conditions necessary, particularly for chronic tests. To improve the outcome for chronic studies with *C. dipterum*, two laboratory studies were conducted. In the first study temperature, food concentration and photoperiod were each varied (4/5 levels) in isolation, with the other parameters fixed for each of the changing treatment conditions. A control scenario was common across all three treatment conditions. In the second study, the organisms were exposed to three different concentrations of Imidacloprid (1, 33, 100 µg/L), and a control scenario, at two differing exposure patterns (24 hour exposures separated for either 2 or 9 days). Some adjustments were made in the design of Study 2, based on the findings of Study 1 and the outcome of a mayfly ringtest. In both studies, body length and instar stage were measured weekly, and mortality and emergence recorded daily. A major finding of the first study was the strong relationship between temperature and growth and development, against the associated effect on mortality (e.g., higher temperature led to faster growth and development, but also higher mortality). Furthermore, a change of the food source used in the second

study showed how periphyton was a superior food source over the vegetarian fish food used in Study 1, with an improvement in the control emergence from 40 to 80%. The second study indicates the suitability of instar development for the assessment of sublethal endpoints. Whereas there was no distinct difference in mortality between the exposure patterns, the Instar Development Index highlighted that the effects were more pronounced in the exposure scenario with the pulsed exposures in quick succession, compared with the longer interval between exposures. These results provide new insight into the culturing of *C. dipterum* and show potential to be used to calibrate/validate DEB modelling implemented in line with EU pesticides RA.

2.05.T-03 Sequential Pesticide Exposure: Concentration Addition at High Concentrations _ Inhibition of Hormesis at Ultra-Low Concentrations

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Sequential pesticide exposure is a common scenario in both aquatic and terrestrial agricultural ecosystems. Predicting the effects of such exposures is thus highly relevant for risk assessment. However, there is currently no information available for predicting the effects of sequential exposure to the same toxicant at both high and low concentrations. Here we exposed one-week-old individuals of *Daphnia magna* to the pyrethroid Esfenvalerate for 24 hours and compared the effects with individuals treated twice with half the concentration after 7 and 14 days. We showed that at the concentrations close to the LC50, both the survival and population growth rates from the two half-pulses were consistent with the concentration addition approach. At low (1/10th to 1/100th of the LC50) and ultra-low concentrations (1/100th to 1/1000th of the LC50), survival was around 100%, while the population growth rate showed a hormetic increase following the one-pulse exposure but not for the two-pulse exposure. We hypothesize that this hormetic effect is due to lower systemic stress (SyS) after pesticide exposure in combination with only one rebound stress pulse. Our study suggests that while the lethal effects of sequential exposure are according to the concentration addition model, the sublethal effects at low and ultra-low concentrations need to consider hormetic effects.

2.05.T-04 Improving Interim Freshwater Guideline Values for Aluminium by Investigating the Effects of Laboratory-Spiked and Field-Collected Water on Aquatic Invertebrates

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Aluminium is a non-essential metal frequently found in aquatic environments from both natural and anthropogenic sources, with no known biological function. In Australia, species protection guidelines for aluminium recognise that pH significantly affects its bioavailability and subsequent toxicity to organisms. Therefore, the 95% species protection value is set at 0.8 µg/L for pH < 6.5, and 55 µg/L for pH > 6.5. Surveys of freshwater bodies around Melbourne, Australia, found that aluminium concentrations often exceed these guideline values, sometimes by several orders of magnitude. The validity of current species protection guideline levels was investigated. This study aimed to assess the effects of acute and chronic exposure to environmentally relevant concentrations of aluminium on two freshwater invertebrates: *Chironomus tepperi*, a non-biting midge, and *Austrochiltonia subtenuis*, a freshwater amphipod, at pH < 6.5 and neutral pH. Additionally, the study evaluated the impact of field-collected water on acute endpoints in these two species. Laboratory exposure results indicated that amphipods were more sensitive than chironomids at pH levels below 6.5, with significant reductions in survival and growth during acute and chronic exposures at 2,500 µg/L. In contrast, a significant reduction in survival for *C. tepperi* was observed only at 5,000 µg/L. Amphipod growth significantly declined at aluminium concentrations of 100 µg/L or higher. Although chironomids appeared smaller at all aluminium concentrations, the reduction was not statistically significant. Interestingly, at neutral pH, chironomids were more sensitive, showing a significant decrease in acute survival at 5,000 µg/L, while amphipods showed no significant impact at any aluminium concentration. Water was collected from five sites based on aluminium concentrations from previous surveys. We predicted that we would observe effects on survival and growth at two sites with the highest concentrations of aluminium, based on the laboratory results. Acute exposure to the field-collected water revealed no significant impact on *C. tepperi* survival but unexpectedly, there was a significant increase in *C. tepperi* growth. This may be related to factors such as water hardness and nutrients. These

findings can be used in the revision of the current interim Australian and New Zealand Aluminium freshwater guidelines, providing a more robust ecologically relevant level of protection for local species.

2.05.T-05 Organophosphate Esters Disrupt Copepods Health and their Associated Microbiome: From a Large Cross-Atlantic Ocean Field Study to Confirmatory Lab Experiments

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Organophosphate esters (OPEs) are widely used as flame retardants, plasticizers, and additives, making them significant emerging contaminants in marine environments. They pose a threat to marine organisms due to their bioaccumulation and toxicity. Copepods play a vital role in aquatic ecosystems through feeding, nutrient cycling, and migration, but their role in the distribution and impact of OPEs remains unclear. Additionally, copepod-associated microbial communities, sensitive to environmental changes, may play a key role in understanding OPE effects. This study aimed to investigate the impact of six OPEs on copepod *Paracartia grani*, focusing on reproduction, bioaccumulation, and microbiome changes under controlled conditions. Furthermore, the findings were integrated with field observations of changes in copepod-associated microbiomes along an Atlantic transect, where OPE concentrations were measured in the water column. For the experiment, copepods were exposed to a mixture of six OPEs (TCEP, TCPP, TNBP, TPHP, EHDPP, and TCMP) at two concentrations: 200 ng/L and 2000 ng/L. Egg production, bioaccumulation, and 16S rRNA gene amplicon sequencing were assessed. Field copepod and water samples were collected from 24 stations along a latitudinal transect from Vigo (Spain) to Punta Arenas (Chile). DNA was extracted for 16S rRNA gene sequencing to characterize copepod-associated microbiome and OPE concentrations were measured in water samples. Exposure to OPEs resulted in a >30% reduction of egg production, regardless of concentration. OPE bioaccumulation in copepods was influenced by the compounds LogKow values, and copepods eliminated OPEs through fecal pellets. OPE exposure significantly altered the copepod-associated microbiome, increasing the relative abundance of Enterobacterales and Pseudomonadales, which were identified as key contributors of OPE exposure. In the field study, OPE concentrations in surface and DCM water layers correlated positively with stations in the northern and northern-central Atlantic Ocean. Similar to the experimental results, OPE concentrations positively correlated with Enterobacterales (*Vibrio*, *Photobacterium* and *Pseudoalteromonas*). In conclusion, OPEs significantly impact copepod reproduction and associated microbiome composition. The findings emphasize the importance of linking laboratory experiments with field studies, using omics approaches, to understand the real-world effects of pollutants on marine ecosystems.

2.05.P Lab and Field Collected Invertebrates and in Situ Studies in Ecotoxicology: Challenges and Opportunities

2.05.P-We071 In Situ Mussel Watch at Volcanic CO₂ Vents: Exploring Ocean Acidification Impacts on Contaminant Dynamics and One Health Risks

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This study presents a "Mussel Watch" field experiment aiming to examine how ocean acidification (OA) gradients affect contaminant accumulation and physiological stress in marine organisms, specifically the Mediterranean mussel *Mytilus galloprovincialis*. Recent findings indicate that organisms inhabiting naturally acidified environments experience considerable energetic and oxidative stress, suggesting potential impacts on their capacity to manage environmental contaminants. This research leverages OA gradients around Ischia, where mussels were monitored over a two-month period to investigate how OA conditions may influence contaminant bioaccumulation and toxicological effects. A central component of this study involves analyzing contaminant concentrations within mussel tissues to explore potential risks in a One Health framework. These contaminant profiles will then expose human cell cultures to the detected concentrations, simulating realistic environmental exposure scenarios. This approach will help assess how OA-related changes in contaminant dynamics could pose broader ecological and human health risks. This study highlights the impact of ocean acidification on contaminant bioaccumulation, physiological stress, and mortality in *Mytilus galloprovincialis*. It shows that acidified conditions, like

those near CO₂ vents, can reduce contaminant accumulation but increase stress and mortality. These findings emphasize the complex interactions between environmental stressors and contaminants, with implications for ecosystem resilience and public health. Further research is needed to understand the mechanisms and long-term effects of ocean acidification.

2.05.P-We072 Unveiling the Toxic Trail: How Methylmercury Accumulates and Impacts Zebra Mussels in Field and the Lab

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Methylmercury (MeHg) bioaccumulation in food webs poses a health risk linked to fish consumption. However, the role of freshwater primary consumers at the base of food web in the Hg cycle remains insufficiently explored. *Dreissena polymorpha*, is a food source for crustaceans, fish, birds, and mammals. This study aimed to assess the bioaccumulation and effects of environmentally relevant dietary MeHg concentrations in *D. polymorpha*. Mussels were exposed by caging in the Seine River where high Hg levels in fish were documented and in controlled laboratory experiments to better understand how *D. polymorpha* accumulates MeHg.

Mussels were caged in the Seine River (Paris) with 1 cage retrieved weekly for 3 weeks. In laboratory, mussels were fed 4 days with microalgae (*Chlorella vulgaris*) pre-exposed to Me²⁺²¹⁰Hg and I¹⁹⁹Hg. Bioaccumulation of MeHg was analyzed in *D. polymorpha*, *C. vulgaris*, and water. Metabolomic, lipid peroxidation, enzymatic activity and transcript levels of antioxidant defense were performed.

In the field, MeHg concentrations and proportions (up to 95%) in *D. polymorpha* increased over time. 13 metabolites in 7 metabolic pathways, including amino acid and energy metabolism, showed significant reductions, suggesting a metabolic shift that could affect the mussel's fitness. While lipid peroxidation, enzymatic activity, and transcript levels of antioxidant and defense were modulated, these changes were not statistically significant.

In the laboratory, MeHg concentrations in *D. polymorpha* increased over time, while its proportion remained stable at 10.3 ± 0.9%. No evidence of Hg methylation or demethylation was detected in the mussels. In *C. vulgaris*, MeHg was predominantly localized in the intracellular compartment, indicating that the MeHg accumulated by *D. polymorpha* likely originated from this fraction of the microalgae. Metabolomic profiles, enzymatic activity, and transcript levels associated with the antioxidant and defense systems showed no significant changes.

This study demonstrated that *D. polymorpha* rapidly bioaccumulates MeHg in both field and laboratory conditions. Laboratory experiments further revealed that MeHg is absorbed from Hg-contaminated food rather than being methylated in the gut. At the low MeHg concentrations tested in the laboratory, no significant biological effects were observed. Future experiments will focus on dose-response analyses across a wider range of dietary MeHg concentrations to better understand its impacts.

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2.05.P-We073 Investigating Primary Producers Buffer Effect Against Mercury Pollution in Two Marine Invertebrate Species

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Human activities have substantially impacted marine ecosystems through the introduction of contaminants. Oxygen release from photosynthetic organisms may support marine animals' aerobic performance in challenging conditions, such as ocean warming. However, the role of oxygen in mitigating pollution effects remains poorly understood. This pilot study investigates whether oxygen supersaturation can alleviate the impact of mercury pollution, simulating conditions of high primary productivity. Experiments were conducted on two commercially important species, the sea urchin *Paracentrotus lividus*

and the clam *Ruditapes philippinarum*. Adult specimens were collected from the Venice Lagoon and exposed to a combination of two oxygen saturation levels (90% as the control and 160%, simulating daily supersaturation) and two mercury concentrations (0 and 1 µg/L, as mercury chloride) for seven days. Biomarkers of oxidative stress, immunosurveillance, and mercury bioaccumulation were assessed in both species, while physiological and behavioral traits were analyzed exclusively for *P. lividus*. Results revealed species-specific responses to mercury under hyperoxic conditions. Notably, clams exhibited reduced mercury bioaccumulation under hyperoxic conditions compared to normoxic ones. In sea urchins, higher mercury accumulation was observed in female gonads compared to males, while no significant effects on physiological and behavioral traits were detected. This study forms part of the RETURN Extended Partnership, funded by the European Union's NextGenerationEU initiative (National Recovery and Resilience Plan NRRP, Mission 4, Component 2, Investment 1.3 D.D. 1243 2/8/2022, PE0000005).

2.05.P-We074 Di(2-ethylhexyl) Phthalate Induced Altered Expressions of Genes Involving in Immune Response, Cellular Defense and Apoptosis in the Mud Crab

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Di(2-ethylhexyl) phthalate (DEHP), an extensively used plasticizer, mainly serves as an additive to render polyvinyl chloride soft and flexible. It is ubiquitously distributed in the aquatic environment and considered an endocrine disruptor chemical (EDC). In the present study, we investigated the potential effects to DEHP exposure on immune responses (interleukin enhancer-binding factor: ILF), cellular defense (heat shock factor 1: HSF1, heat shock protein 70: HSP70, HSP90, HSP40, HSP83, HSP67B2, HSP60, and HSP21), and apoptotic responses (BAX, p53, Bcl-2) in the mud crab *Macrophthalmus japonicus*. To do this, we characterized the partial sequences of HSF1 and ILF. Phylogenetic relationships were analyzed in crustacean HSF1 and ILF genes. DEHP exposure induced increase of the HSF1 and ILF gene expressions. In addition, it changed the expression levels of seven HSPs (upregulation of HSP70, HSP40, HSP67B2, HSP83, and HSP90 and downregulation of HSP21 and HSP60) in the gills and hepatopancreas of *M. japonicus* exposed to DEHP. These expressions of HSPs were conjugated with responses of HSF1 and ILF. Apoptotic signals of BAX, p53, and Bcl-2 were altered in both tissues of *M. japonicus* at the protein levels by DEHP exposure. These results suggest that long-term exposures to DEHP could trigger the unbalance of immune system and cellular homeostasis, and finally increase of apoptotic cells leading to mortality of the mud crab.

2.05.P-We075 Effects of Neonicotinoids Exposure on the Blue Crab (*Callinectes sapidus*): Bioaccumulation, Neurotoxicity, and Behavioral Implications

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Neonicotinoid insecticides may play an important yet underestimated role as stressors of coastal ecosystems, which may potentially contribute to the decline in marine crustacean populations. Phylogenetically, crabs are more closely related to the insects targeted by neonicotinoids than fish or mollusks. Thus, this study investigates the effects of two widely used agricultural neonicotinoids Acetamiprid and Imidacloprid on the blue crab (*Callinectes sapidus*), an ecologically and economically important estuarine predator, prey, and fishery species. Microcosm experiments were conducted to assess the link between neonicotinoid exposure and bioaccumulation, and their role to produce neurotoxicity (measured through the acetylcholinesterase - AChE- activity), and behavioural effects (measured through endpoints such as aggressivity reflex, eyestalk responses, righting time, and foraging activity), which may have ecologically relevant implications for predator avoidance and survival. In the experiments, subadults and adult crabs were offered gelatin seafood pellets (approximately 8% of the individual's wet weight) every three days, contaminated either with Acetamiprid (nominal concentration of 3 µg/g wet weight) for 14 days, or and with Imidacloprid (nominal concentration of 1 µg/g wet weight) for 23 days. Each treatment and control group consisted on 12 individuals with a balanced sex ratio (1:1). Preliminary results showed a significant decrease in feeding frequency, along with notable behavioural symptoms such as chelae spasms and sustained spastic leg movements during stationary periods in the Acetamiprid-exposed group. Acetamiprid and Imidacloprid tissue distribution as well as AChE activity levels across various tissues will be presented. The results will be discussed with the aim of highlighting the potential implications for the survival and ecological role of blue crabs in estuarine ecosystems, as well its potential use as target species for pollution assessment in field studies.

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2.05.P-We076 The Amphipod *Marinogammarus marinus* in Ecotoxicological Studies: Evaluating Responses to Environmental Stressors

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Amphipods, a globally distributed order of crustaceans, are predominantly found in aquatic habitats, especially marine environments. They are considered good indicators of environmental health due to their sensitivity to a wide range of pollutants and their ecological importance in the food web. Despite this, few species have been used as test organisms in ecotoxicology, compared to other invertebrates. In this work, responses of the amphipod *Marinogammarus marinus* (Leach, 1816) to various stressors are evaluated and its potential as a model species is considered, as it revealed sensitivity to all stressors tested. *M. marinus* is an epibenthic intertidal macroinvertebrate, inhabiting European Atlantic estuaries and coastal areas. As a non-model organism, it has been subject of limited research, although its potential has been suggested. The species is easy to maintain in laboratory, with periodic reproduction and high reproductive rates, and known life cycle. Specimens were field collected in Portugal, and laboratory cultures were established. In this study, *M. marinus* was exposed to metal contamination, toxic microalgae and temperature, with acute toxicity thresholds being determined. Amphipods exposed to cadmium presented, after 96 hours, an Lc50 (half maximal lethal concentration) of 0,725 mg/L (0,466 1,283) for juveniles and 0,871 mg/L (0,442 1,655) for neonates. For copper exposure, no mortality was observed for juveniles (Lc50>128 µg/L), and only limited mortality occurred in neonates after 96 hours. Regarding microalgae, juveniles were exposed to live strains of *Ostreopsis* cf. *siamensis* and *Prorocentrum lima*, two toxin-producing dinoflagellates. After 48 hours, the amphipod mortality increased with exposure to *O. cf. siamensis*, comparing with the controls, with an Lc50 of 2143 cells/mL (1833 2500). The effect of *P. lima* in mortality could not be determined (Lc50>241 800 cells/mL), although there were effects in immobilization, with an Ec50 (effective concentration) of 111 851 cells/mL (80 913 154 291). Increased temperature was tested in juveniles exposed to *P. lima* for 96 hours. Elevated temperatures (24°C) increased mortality in controls after 72 hours, with the effects of toxic microalgae also intensified at 24°C. These findings contribute to the knowledge on the sensitivity of *M. marinus* to environmental stressors such as pollution and climate-related changes and highlight the use this species in laboratory ecotoxicological experiments.

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2.05.P-We077 Effects of Gadolinium Exposure at Environmentally Relevant Levels in two Freshwater Invasive Species

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Freshwater ecosystems are vital for biodiversity and human well-being, but they are threatened by pollution, overexploitation, habitat degradation and invasive species. In particular, human activities are closely linked to water pollution, with pollutants from agriculture and urban waste entering these ecosystems. Among emerging contaminants, gadolinium (Gd), used as a contrast agent in magnetic resonance imaging (MRI), has been of concern since the 1990s. After excretion, Gd passes through

wastewater treatment and enters aquatic systems, and its presence has been documented worldwide, particularly in densely populated urban areas of countries with advanced healthcare systems. This study investigated the effects of Gd on two invasive freshwater species in Italy: the Louisiana red crayfish (*Procambarus clarkii*) and the zebra mussel (*Dreissena polymorpha*), both effective indicators of pollution. A total of 45 adult of *P. clarkii* and 450 of *D. polymorpha* specimens were exposed to four environmentally relevant concentrations of commercially sourced Gd (Gd₂O₃ - Inorganic Ventures, Christiansburg, VA, USA): 0 µg/L, 0.1 µg/L, 1 µg/L, 10 µg/L and 100 µg/L for 14 days. After exposure, target organs were collected for oxidative stress analysis using biomarkers such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), lipid peroxidation (LPO) and protein levels. For *P. clarkii*, oxidative stress was evaluated in three tissues: muscle, hepatopancreas, and gills. For *D. polymorpha*, three different tissues were analyzed to assess oxidative stress responses: foot and mantle, digestive gland, and gills. The analyses revealed distinct oxidative stress responses in *P. clarkii* and *D. polymorpha*, with enzymatic defense activation dependent on Gd concentration. These findings are important for understanding how aquatic species respond to environmental stressors and for assessing ecotoxicological risks associated with Gd. Moreover, the use of invasive species in research could also support strategies aimed at containing these organisms, thereby contributing to biodiversity conservation initiatives.

2.05.P-We079 Dynamics of Zooplankton Biodiversity and Abundance in the Freshwater Lentic Mesocosm System Located in Southern Poland

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Outdoor mesocosm systems are used in risk assessment posed by introduction of plant protection products, wastewater and other chemicals into water and soil. Mesocosm studies are performed under field conditions and mimics ecosystems in natural environment. Therefore, it allows to predict, understand and confirm the effects of a chemical on selected, closely related aquatic ecosystem parameters. The analysis of zooplankton populations in mesocosm studies could be crucial when assessing the effects of insecticides. Due to the field nature of the study, the aim of the presented project was to analyse the seasonal variability of selected species and genera of zooplankton during one summer season as well as selected groups of zooplankton in relation to selected parameters. The test was performed in stainless steel tanks, settled in the ground (about 2400 L each tank), situated in Southern Poland. The tanks were supplied with an appropriate amount of sediment and water and inoculated by adding sediment and water from natural reservoirs. Sampling was carried out from May 2024 to August 2024. Project included qualitative and quantitative zooplankton analysis, physico chemical analysis of water, e.g., temperature, pH, nitrites, nitrates, phosphate, ammonium, chlorophyll-a in water and Total Organic Carbon. During observations, the number of taxa verified to at least genus level ranged from 34 to 48 depending on the tank. The total abundance of taxa indicated changes in the number of individuals from subdivision Copepoda, superordo Cladocera and phylum Rotifera. Copepoda were represented throughout entire studied period mainly by *Mesocyclops leuckarti* while Cladocera were mainly presented by *Daphnia longispina* and *Chydorus sphaericus*. In phylum Rotifera the most abundant taxa were represented by genus *Polyarthra* sp., species *Anuraeopsis fissa* and *Keratella quadrata*, while *Euchlanis dilatata* and *Hexarthra mira* appeared only in July and August.

Shifted levels of Copepoda individuals numbers were observed during chlorophyll-a increases, which were recorded from the middle of July and emerged in relation to the phytoplankton blooming by e.g., *Dolichospermum* sp., *Microcystis* sp., *Trachelomonas* sp. or *Ceratium hirundinella*. The increase in chlorophyll-a level was associated also with a decrease in turbidity.

The obtained data may constitute a database for interpreting the risk experiments results, and may also implicate the processes of designing mesocosm field studies.

2.05.P-We080 Do Mesocosm Studies Still have a Future as Higher Tier Risk Assessment Tool?

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Mesocosm studies are recognised in the EU as the reference tier in aquatic risk assessments of plant protection products (PPPs) for algae, macrophytes and invertebrates. They have been used for over 30 years to analyse the effects of PPPs on aquatic populations and communities. However, the aquatic guidance document is now more than 10 years old, with the last multi-stakeholder workshop on the use of mesocosm studies (CLASSIC) held 25 years ago. Several critical issues have been raised in recent years, both from an industry and regulatory perspective. These include the acceptability and criteria for ecological

recovery, worst-case nature of tested exposure scenarios versus predicted exposure profiles, representativeness of lentic systems for stream communities, approaches to conducting studies focusing on macrophytes and/or phytoplankton, details on statistical methods, e.g. the use of MDDs, and finally, the use of results from multiple mesocosm studies within a risk assessment. The aim of this poster is to outline the critical issues and to stimulate interest among tripartite stakeholders in setting up a SETAC working group and/or organising a SETAC workshop to prepare contributions as input for consideration in the future refinement of the aquatic guidance document.

2.05.P-We081 Maximising Species Abundance Counts in Mesocosm: What Traps Work Best?

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Aquatic, freshwater, mesocosms are designed to represent edge of field water bodies, creating a habitat that will support communities of aquatic macrophytes, algae, zooplankton and macroinvertebrates in a contained system. Macroinvertebrates and emergent insects play an ecologically important role in the ecosystem by performing essential biological and ecological functions and are often considered key sensitive taxa in higher tier studies. However, generating reliable data and endpoints for these groups can be difficult as they are naturally found in low and variable abundances.

Macroinvertebrates and emergent insects are diverse assemblages of organisms, existing all parts of the water column, in and on the sediment, and emerging from the surface of the water during adult stages. How to target collecting these organisms is, therefore, highly dependent on their life stage and the time of year. Macroinvertebrates also require safe places to shelter to avoid predation and thrive until reproduction or emergence. The ability to deploy multiple traps within a mesocosm study targeted to specific environmental niches, makes these test systems ideal for investigating the effects of stressors on this group of organisms. Whilst it may not be feasible to assess the abundance of these mesocosms as a whole, and there are limitations regarding the sample processing time and size, type and number per mesocosm, the use of specific traps allows the collection of these organisms in a targeted and efficient approach designed to maximise abundance counts. The decision of trap design and selection to achieve the best endpoints should give consideration to multiple influences, including the stressor being investigated (e.g., the GAP for a PPP), the species, and the most sensitive life stages, as well as seasonal influences during the in-life phase of the study.

The aim of this poster is to share our expertise on the selection, design and deployment of macroinvertebrate and emergent insect traps in mesocosm studies, to achieve robust and reliable endpoints. We hope that by sharing our considerations for trap selection and endpoint evaluation we open discussions on the representativity of mesocosm studies for these taxa, and help to optimise the experimental design of future mesocosm studies focussed on macroinvertebrate and emergent insects.

2.05.P-We082 Mesocosms Offer a Robust Higher Tier Test System with High Repeatability Despite Biological Variability: A Case Study With Diflufenican

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Using the example of the herbicide diflufenican we would like to demonstrate the robustness of mesocosm studies despite their known higher biological variability compared to standardized single species tests. For diflufenican three different mesocosms exist. Based on lower tier studies, algae were the most sensitive group and thus the studies focused on direct effects on phytoplankton, periphyton and indirect effects on zooplankton and grazers. They were performed in the years 2007, 2010 and 2021 at three different test sites in Germany and the Netherlands. The test design of the three studies differs as well as the test periods and by this the weather conditions under which the three studies have been performed. Exemplarily, water temperatures differed between the first study (March - June 2007) with temperatures between 5 and 20°C and the latest study (June - August 2019) with water temperatures between 15.4 and 25.5°C.

The fact that three mesocosm studies exist for one single test item allows the evaluation of this study type with respect to the consistency and robustness of results. Similar effect thresholds (NOECs) were observed in the three mesocosm studies varying by a factor of about 3. The difference observed is within the factor chosen between two test concentrations used in these studies. Two of the three studies were performed before the AGD (EFSA, 2013) was published and did not fulfill the new requirement of a minimum of 8 potentially sensitive species with acceptable MDD values. Nevertheless, these two studies resulted in effect thresholds fully in line with the latest study (2021) which fulfilled the requirements of

the AGD and resulted in an effect threshold between the ones of the two older studies. The example of these three studies investigating diflufenican demonstrates that mesocosms are a robust and suitable tool to be used in the environmental risk assessment. The comparison of the three studies demonstrates as well, that even older studies not completely fulfilling the current requirements have a scientific value and should be considered in a weight of evidence approach within the aquatic risk assessment for pesticides. In cases where more than one mesocosm exists it should be evaluated whether a combined evaluation is possible to fulfill the requirements of the AGD on 8 potentially sensitive species.

2.05.P-We083 Using Multiple Exposure Techniques to Identify the Sub-lethal Effects of Recycled Water Exposure on Shrimp

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Globally, sewage wastewater is treated and repurposed for human consumption, agriculture, irrigation and the environment. Recycled water released into aquatic ecosystems still contains pollutants including pharmaceuticals, pesticides and metals. Laboratory studies have demonstrated that elevated concentrations of individual pollutants can be lethal to aquatic macroinvertebrates. In waterways, these organisms are exposed to lower concentrations of multiple pollutants over extended periods. Understanding how recycled water impacts aquatic ecosystems is crucial for preserving biodiversity and ecosystem health, yet it presents several challenges. Firstly, pollutants exhibit complex interactive and cumulative effects. Secondly, the composition and concentration of pollutants in waterways are highly dynamic. Finally, sensitivity to pollution varies significantly between taxonomically similar animals and may vary under different exposure conditions. My research will use field-collected water containing environmentally relevant concentrations of pollutants. Exposures will be sublethal to identify any stress response in shrimp. Experiments will be repeated with different species and populations of shrimp to test for family-level variation in sensitivity to aquatic pollution. Shrimp have been selected due to their ecological significance in aquatic ecosystems globally. A series of long-term exposures will be conducted under laboratory conditions and replicated in situ at a wastewater treatment facility. This will facilitate a comparison between stable laboratory conditions and variable field environments. Biomarkers and growth endpoints will be used to quantify the sublethal effects of pollution exposure. This study aims to bridge the gap between controlled laboratory and dynamic field conditions. By examining the sub-lethal responses to chronic exposure, we will gain a more nuanced understanding of how pollution affects aquatic ecosystems. Exposing an ecologically important species with a broad habitat range and global distribution ensures that the findings are widely applicable. Ultimately, we hope this research will have application in aquatic biomonitoring programs worldwide.

2.05.P-We085 Analysis of Phospholipids Modulation by Perfluorononanoic Acid on the Freshwater Gastropod *Physella acuta*

Ignacio Lopez-Alonso, Jose-Luis Martinez-Guitarte and Maria-Angeles Garcia-Fernandez, Universidad Nacional de Educacion a Distancia, Spain

Perfluoroalkyl substances (PFAS) emerge as persistent pollutants in different ecosystems, showing various effects. Freshwater ecosystems act as sinks for PFAS, affecting the flora and the fauna. There is a lack of data about these compounds' effects on living organisms, especially invertebrates. *Physella acuta* is a freshwater gastropod at the base of the food web. To explore PFAS's effects on this snail, perfluorononanoic acid (PFNA) was selected and used to expose the animals for one week. The results of the phospholipid profile analysis by nuclear magnetic resonance (NMR) are presented here. Phospholipids were extracted with a 2:1 water: chloroform mixture (v/v) followed by a second round with Folch methods. The phospholipid profile was analyzed by 1D 31Phosphorus NMR. The results showed a change in the profile depending on the PFNA concentration, with new signals indicating a change in the proportion of different phospholipids. Additional research is needed to elucidate the mechanisms involved in the alteration produced by PFNA, but combining 1D 31Phosphorus NMR with gene expression will help to understand the molecular pathways affected. Finally, 1D 31Phosphorus NMR offers a simple method to analyze the changes in phospholipid profile by toxicants on invertebrates, opening new possibilities for elucidating the mechanisms involved in the toxicity of PFAS.

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2.05.P-We086 Acute Oral Exposure of the Non-target Foliar-dwelling Arthropod *Chrysoperla carnea* (Stephens) Using Artificial Diets

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Currently there are different test protocols to assess the risk of a plant protection product on non-target foliar-dwelling arthropods. However, these protocols usually propose residual exposure methods to test substances that may be ineffective for assessing the risk for microbiological products acting by ingestion. This work aims to propose new testing methodologies for microbiological substances, based on an oral exposure mode, which allow an accurate risk assessment on the aphidophagous predator, *Chrysoperla carnea* (Stephens), and are feasible for laboratories. For this purpose, several chemicals, with known effects by ingestion, were evaluated in order to validate the methodology.

Two to three day old larvae were acutely exposed during the first 48 hours to the test substance incorporated into an artificial diet. Different concentrations were tested to evaluate the pre-imaginal mortality and calculate the LC50 value. Our work provides an adequate acute oral exposition methodology as a first step for the development of future risk assessment trials for microbiological plant protection products with an ingestion mode of action on *C. carnea*.

2.06 Impact of Airborne Pollutants on Environmental Health and Biodiversity

2.06.T-01 Silent Threat: The Impact of Airborne Pesticides on Biodiversity

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Agricultural pesticide drift is a hidden threat, wherein the pesticides take to the air, extending well beyond the target application site and contaminating surrounding environments. While direct exposure to pesticides has been a common and well-informed subject of research, fallout from pesticide drift is not as well understood but nonetheless causes alarm. Recent studies have revealed an unsettling trend where, over time, populations of different species are clearly in decline due not in a small part to airborne pesticides. These chemicals act to promote the decline in biodiversity by their effects imposed on non-target organisms such as plants, insects, fungi, and many other forms of organisms living near habitats or even in ecosystems quite far away from where this might be used. Still, the full scale of their influence has not yet been determined. In the current study, the authors reviewed the most recent knowledge about the possible contribution of air-borne pesticides to the decline in biodiversity and discussed mitigation methods. The results indicated critical knowledge gaps and, therefore, a need for urgent measures to protect environmental health. Further studies should realistically be field-oriented, investigating multiple applications of pesticides and their interactive effects with flora and fauna in space and time.

2.06.T-02 Multiphase Transformation of Nitrogen Containing Organic Compounds in the Indoor Environment

Chen Wang, Southern University of Science and Technology, China (Mainland)

A wide variety of nitrogen containing organic compounds are present in indoor environments from the use of chemical products and emissions from occupant activities, including cooking, smoking, burning, etc. For example, organic amines including aromatic secondary amines and their derivatives, and azo dyes were detected in various dust substrates indoors. Most of these organic compounds are semivolatile, which can be easily sorbed to indoor surfaces such as glass, clothes, and furniture with the settlement of particles and gases indoors. In the indoor environment, there are many reactive oxidants, including ozone (O₃), nitrous acid (HONO), and during cleaning, reactive chlorine species (hypochlorous acid - HOCl, chloramines - NHCl₂, NH₂Cl, chlorine gas - Cl₂, etc.). Exposure of the surface sorbed organic compounds to these oxidants can lead to degradation of the organic contaminants and formation of potentially harmful transformation products both in air and on surfaces. In this talk, case studies involving multiphase transformation of nitrogen containing organic compounds in the indoor environment and its impact on indoor surface and air composition will be discussed. We identified the specific molecular identity of the products based on high-resolution mass spectrometry analysis. We confirmed that nitrosamines and other transformation products of organic amines are produced through heterogeneous reactions on surfaces in a real indoor environment. Transformation of organic compounds upon exposure to ozone and reactive chlorine species will also be presented.

2.06.T-03 Toxicity Assessment of Airborne Ultrafine Particles: Role of Transport Emissions

Benjamin Pina, IDAEA-CSIC, Spain

Airborne quasi-ultrafine particle samples were collected from different outdoor sites in Barcelona (NE Spain, 35 samples) and the Valencia subway (about 400 km south of Barcelona, 3 samples). Locations and schedules were designed to cover cold and warm seasons and to represent the impact of different types of transport (cars, trains, ships, and planes). PTFE filters were extracted with a mild organic solution (methanol:dichloromethane 1:2) and the extracts were used to test toxic effects in human cell lines (Induction of reactive oxygen species, inflammatory response) and in zebrafish embryos (expression of xenobiotic response-related genes, *cypl a1*, *gsa1* and *ha1*). The highest toxicity values were detected in sites receiving car and/or ship emissions, with maximums during the cold season. Chemical analyses followed by correlation and source apportionment analyses identified PAHs, combustion engines, and biomass burning emissions as the main drivers of the observed toxic effects. Although traffic restrictions and car emission limits significantly reduce organic pollutants in urban air, a potential ban on all combustion processes will be the only effective way to eliminate airborne toxicity in our cities.

2.06.T-04 Considerations for Using Moss as a Biomonitor for Airborne Microplastics

Richard Cross¹, Sarah Roberts² and Felicity Hayes², (1)CEH, United Kingdom, (2)UKCEH, United Kingdom

Mosses have been proposed as a passive bio-monitor for the deposition of airborne microplastics. European moss surveys have long been reported on by the Working Group on Effects (WGE) of the Convention on Long-range Transboundary Air Pollution (LRTAP). The first moss survey at the European scale was conducted in 1990, with subsequent surveys at 5-year intervals. As part of the 2020 survey, a new sampling and analysis protocol was established to include an assessment of microplastics in the UK. To establish whether moss may be used as an effective bio-monitor for MPs, it is essential to consider the resolution and power achievable in the data, any confounding influences in monitoring design and the reproducibility of a suitable quantification method. Applying these criteria to the 2020 ICP Vegetation moss survey of 52 sites across the United Kingdom, a novel method using turbulent flow displacement to extract microplastics from large moss samples (~10 g material) was optimised. This balanced achieving low background contamination whilst also ensuring high recovery of microplastics from the moss superstructure. Extracted samples were analysed using micro-Fourier Transform Infra-Red spectroscopy (μ -FTIR).

Almost all moss samples were found to contain microplastics >25 μ m in size, above limits of detection. Maximal differences between sites on a particle number per gram moss was ~35-fold. Six major genus of moss common to the United Kingdom were collected, representing a variety of mat and cushion phenotypes. Both moss characteristics but also variables associated with land use and geographies were used to explore possible influences over differences in polymer composition and abundance across the UK. Practical recommendations around sampling design for the use of moss as a biomonitor of microplastics will be critically discussed and recommendations made for future sampling design.

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2.06.T-05 Health Quality of Urban Particles According to their Load in Endocrine-Disrupting Compounds and Associated Toxic Potential

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The harmful effects of air pollution are partly linked to atmospheric particles (PM), due to their size, but their composition can also play a role. Indeed, they can adsorb semi-volatile organic compounds (SVOCs) with suspected or proven endocrine-disrupting (ED) properties. In order to better understand the link between contamination of PM in ED compounds and associated biological effects, 3 urban sites were selected in Paris region (France). On each site, 3 classes of PM were collected (PM_{2.5}, PM₁₀ and TSP), at 2 contrasting seasons and 62 SVOCs were investigated in the PM extracts. The estrogenic and dioxin-like potential were analysed by in vitro bioassays. The results of this work highlighted the highest contamination of PM_{2.5} in accordance with the toxic potential of their bounded-organic contamination. A non-monotonic dose-response effect was observed for estrogenicity of some PM organic extracts. These data allowed us to understand the complexity of the characterization of the health hazard linked to PM according to their size, their origin and in relation to the molecules they adsorb.

2.06.P Impact of Airborne Pollutants on Environmental Health and Biodiversity

2.06.P-Th035 Analytical and Toxicological Evaluation of Residential Closed Combustion Waste Burning

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In this work, we carried out controlled combustion experiments in a residential iron test stove, where specific types of household plastic waste were co-fired along with conventional solid fuels in order to mimic residential waste burning and to analyze the analytical and toxicological characteristics of the emissions. The eco-, cyto- and genotoxic effects of emission samples from closed combustion of legal solid fuels (black locust, oak, black coal, brown coal, briquettes) and various household plastic waste types (polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyurethane (PU)) were investigated. The flue gas emissions were collected in MilliQ water filled impingers and the samples underwent subsequent chemical and toxicological examination.

The total carbon (TC) content was determined, and PAH analysis was carried out by GC-MS. Ecotoxicity of the undiluted samples were measured by *Vibrio fischeri* bioluminescence inhibition assay and the boar sperm motility inhibition test were used for quick screening of toxic effects. Flow cytometric live/dead ratio and genotoxicity with alkaline comet assay were tested on A549 lung epithelial cells using 50, 25 and 5 mg Cdm-3 dilutions.

The findings of the ecotoxicity and boar sperm inhibition tests were categorized as toxic to highly toxic in every sample and showed a strong correlation with the total carbon concentration and PAH content. Cytotoxicity experiments show that emissions from co-incinerating garbage have more harmful impacts than emissions from burning just approved solid fuels. Following a 24-hour exposure period, samples from PP, PE, PET and PS incineration diluted to 50 mg Cdm-3 showed a considerably higher proportion of dead cells than the control. PP, PE, and PET samples showed significant levels of DNA fragmentation, but PS, black-, and brown carbon samples also showed minor genotoxic effects. The toxicity results were well correlated with the PAH content of the samples.

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2.06.P-Th036 Prolonged Exposure of Emerging Contaminant-Phthalate Ester on Human Lung Cells: Cell Respiration Inhibition and Joint Toxicity Prediction

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Phthalate esters (PAEs) such as dimethyl phthalate (DMP), diethyl phthalate (DEP), and di-n-octyl phthalate (DOP) are synthetic chemicals used as solvent stabilisers, and plasticisers in commercial and industrial products, and are classified as priority contaminants of emerging concern (CECs) or new and emerging pollutants (NEPs). Humans are at constant risk of exposure to these toxic PAEs, but their regulations in the air remain limited. PAEs have been detected in inhalable particles such as PM_{2.5}; however, their individual and combined effects on human lung cells beyond 48 h exposure remain unclear. This work evaluated the individual and combined toxicity of prolonged exposure (up to 168 h) to environmentally relevant concentrations of DMP, DEP, and DOP (i.e., 10 ?M - 1000 ?M) and the persistent organic pollutant (POP) bis-2(ethyhexyl) phthalate on Calu-3 human sub-bronchial gland cells. Individual exposure to the tested PAEs yielded the following maximal inhibitory concentration (IC₅₀) at 24 h (4462 ?M DMP, 2049 ?M DEP), 72 h (3068 ?M DMP, 927 ?M DEP), and 168 h (418 ?M DMP, 106 ?M DEP, 343 ?M DEHP, 400 ?M DOP). The experimental results were predicted by the concentration addition (10 ?M - 1000 ?M) and independent action (100 ?M - 1000 ?M) joint toxicity models after 72 h exposure to the DMP-DEP mixture. Toxicity unit calculations predicted a difference in mixture interactions (e.g., synergistic and antagonistic) at different exposure time points for the DMP-DEP mixture. Exposure of live cells to individual or binary mixtures of PAEs showed that cellular oxygen consumption decreased as concentrations increased, indicating toxicity and inhibition of mitochondrial respiratory flux. Results of this work suggest that the currently set workplace air limit of 5 mg/m³ for DMP, DEP, and DEHP (24 h average) by UK regulations could be toxic to human lung cells and may need to be reviewed and updated. More work is needed to understand the toxicity mechanisms of PAEs on

human lungs to facilitate more accurate risk assessments and to set regulations on their atmospheric and workplace air concentrations.

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2.06.P-Th037 Investigating the Allergenic Potential of Different Pollen Species in Terms of Their Cell Viability, ROS Measurement and Chemical Modifications on Exposure to Air Pollutants

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Airborne pollen may lead to severe allergies such as asthma, pollinosis, rhinitis, and lung inflammation. Australia and the UK have one of the highest prevalence of registered allergic disorders (Idrose et al. 2020). Climate change induced extreme weather occurrences, fluctuations in temperature and relative humidity, can influence the transport, dispersion and interaction of pollen allergens and air pollutants (Tran et al. 2023). In an urban setting, presence of pollutants can influence the allergenic potential of pollens via chemical modifications of allergenic proteins and thereby altering their immune response. The cellular mechanisms involved in physical barrier disruption and the influencing protein modifications underlying respiratory exacerbations are not fully understood (Bradbury et al. 2022). Hence, in current research, cellular response of Calu-3 lung epithelial cells has been assessed. The overall aims of this work are to (1) to assess the allergenic potential of different pollen species by assessing their cell viability and oxidative stress (2) to investigate the protein modifications in different pollen species upon exposure to air pollutants.

In this work, Grass, Pine, She Oak and Acacia were evaluated. Cell viability was determined by MTT assay Reactive oxygen species (ROS) measurements were conducted using 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) probe. Cells were treated with pollen protein extracts for 24 h for cell viability assay. For the protein modifications, study will be conducted using Liquid chromatography mass spectrometry (LCMS) and to assess structural changes before and after the exposure to pollutants.

Cell viability results for Calu-3 cells showed a varied concentration dependent response among all species with lowest viability for pine pollen followed by grass pollen. Rate of ROS production was highest for grass pollen. The increase in rate of ROS generation was in concentration dependant manner. ELISA analysis of Ph1 p 5 allergen concentration in grass pollen suggested increase in concentration with increasing extract concentration. Additionally, the impact of anthropogenic pollutants on the protein modifications will be discussed. It is hypothesised that with rising levels of atmospheric oxidants, pollen proteins can undergo modifications (oxidation, nitration, cross linking,) and the formation of resulting complex could be the biomarker for oxidative stress in the lung cells.

2.06.P-Th038 Investigating Airborne Pesticide Deposition Using Trace Analysis in Precipitation, Soil, and Agricultural Plants

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Airborne pesticide transport and deposition, e.g., from agricultural applications, is an underinvestigated source of environmental contamination with potential ecotoxicological relevance for off-target organisms. Furthermore, this leads to pesticide transport from conventional to organic farming, resulting in increasing concerns over pesticide exposure by consumers.

Therefore, the German Federal Office of Consumer Protection and Food Safety (BVL) funded a study to investigate the airborne deposition of relevant pesticides or pesticide metabolites on plants and soil, as well as in rain precipitation, with the aim of comparing the amounts of these substances deposited in the respective compartments. This study is intended to solve possible practical problems for a planned nationwide monitoring of airborne pesticide transport in Germany.

The study was performed on a field belonging to LANUV NRW in Essen, Germany. The experimental setup consisted of a bulk collector to capture rainfall. For the soil, a test soil was used that had no intentional pesticide use during the last 5 years. A soil layer was filled into plastic boxes, divided by a

fleece to collect percolation water. In other plastic boxes of the same surface area, each five pre-grown kale plants were planted. The test was set up over a 28 days long exposure period from June to July 2024 to capture airborne deposited pesticides on soil and kale as well as in rainfall precipitation. This study further included method development, validation and trace analysis of ten pesticides/metabolites in water, soil and kale samples. The target analytes were selected based on findings in a pre-study in water samples from bulk collectors or based on typical application during the time of study performance. Analyses were performed by U(H)PLC-MS/MS including isotope-dilution analysis with limits of quantification in the low ng/L and ng/kg range, respectively. The analytical results from the three compartments water (bulk collector), soil (including percolation water) and kale were related to their collection surface, resulting in ng/m² deposition values. This comparison allows to find the most suitable collector for airborne pesticide deposition that can be used in future monitoring studies. Furthermore, the results shed light on the extent of pesticide deposition onto non-target areas during the main application time of PPPs in early summer in Germany.

2.06.P-Th039 Development of Evaluation Methods and Monitoring Data Analysis for External Pollutant Ingress into Vehicles During Driving

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Globally, people spend about 5% of their day inside vehicles. Despite this relatively short duration, exposure to pollutants in vehicles can constitute a significant portion of daily exposure due to the high concentrations of surrounding pollutants.

The UN WP.29 is actively developing technologies to harmonize VIAQ (Vehicle Indoor Air Quality) evaluation methodologies. While guidelines exist for evaluating emissions from vehicle interior materials, no international standard addresses the inflow of outdoor pollutants into vehicles. This study aims to draft and refine a VIAQ evaluation method through real-road tests.

Pollutants entering vehicles during driving include atmospheric pollutants, exhaust emissions, and non-exhaust traffic-related emissions. Based on a review of regulations and research, PM_{2.5}, PN, NO, and NO₂ were selected as target pollutants. Given that indoor air quality is influenced by outdoor air levels, pollutants were measured simultaneously inside and outside vehicles using two identical devices equipped with isokinetic sampling probes and pumps to ensure reliability.

Real-road driving tests required developing route selection methods, including urban driving followed by highway driving, with each segment spanning at least 16 km. HVAC modes, another influencing factor, were classified as recirculation mode (RC), outdoor air intake mode (OA), and automatic mode (Auto). The average indoor concentrations of PM_{2.5} and NO₂ were lowest in RC mode and highest in OA mode, while NO concentrations were lowest in RC mode and highest in Auto mode. Cleaning efficiency was highest in RC mode for all pollutants, followed by Auto and OA modes.

Analyzing pollutant concentration data alongside external factors revealed significant correlations between indoor pollutant levels and factors such as season, outdoor concentrations, and HVAC conditions, with weaker correlations observed for powertrain types.

This study has limitations, including its focus on weekday daytime hours, which excluded rush hour conditions, and the omission of driver habits or experimental personnel effects in the analysis. Further studies with diverse regions and larger datasets are necessary to reduce biases and generalize findings. Despite these limitations, this research identified external air quality and other influencing factors affecting vehicle indoor air quality during driving, providing foundational data for developing VIAQ evaluation methodologies.

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2.06.P-Th040 Multiple Biomarkers Assessment in the Gill and Blood of Nile Tilapia Induced by Settleable Atmospheric Particulate Matter from Metallurgical Industries

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Settleable atmospheric particulate matter (SPM) released by the metallurgical industries consisted of carbon and a mixture of metals that in contact with aquatic environment disaggregated in metal/metalloids

and metallic nanoparticles. They may be absorbed by fish affecting homeostasis. Nile tilapia, *Oreochromis niloticus* were exposed to 0, 10, 100 and 1000 µg SPM L⁻¹, for 96 h and the bioaccumulation of metal/metalloids and metallic nanoparticles in the gills were evaluated using inductively coupled plasma mass spectrometer (ICP-MS) and transmission electron microscopy and energy-dispersive spectroscopy (EDS). Blood variables, genotoxicity and plasma ions were analyzed in blood; the enzymes related to ion transport and enzymes and molecules from antioxidant defense system and oxidative stress as well as histopathological alterations in gills were evaluated. Metal/metalloids bioaccumulated in the gills of at different concentrations being Fe>Zn>Sr>Al at highest concentrations. Nanoparticles containing Fe, Cr, Al, Cr and Si were identified in gill and blood cells. The activity of Na⁺/K⁺-ATPase, H⁺-ATPase were unchanged and carbonic anhydrase decreased after exposure to 1000 µg SPM L⁻¹. Plasma Na⁺ and K⁺ were unchanged but Cl⁻ content decreased after exposure to 1000 µg SPM L⁻¹. The biotransformation enzyme of phase II, and the antioxidant enzyme catalase increased in the gills of fish exposed to 100 µg SPM L⁻¹. Reduced glutathione increased in all exposure concentrations of SPM and metallothionein increased at the highest SPM concentration. Lipid peroxidation was unchanged. However, filament epithelium hyperplasia, lamellar epithelium hypertrophy, ionocytes hypertrophy and mucous cells proliferation were the most frequent changes in the gills resulting in the increasing the histopathological alteration indexes in fish exposed to 100 and 1000 µg SPM L⁻¹. SPM water contamination increased the metal/metalloids dissolved in water as well as metallic nanoparticles which are absorbed inducing changes in the blood cellular defense system, ionic regulation, and has genotoxic potential. The changes in the gills evidenced a response to reduce metal absorption present in the SPM and overcome ionic disturbance.

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2.06.P-Th041 Analysis of Emissions From Brake Abrasions in Rail Traffic

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In transportation, non-combustion-related emissions are becoming more relevant due to the decline of combustion-related emissions. Although rail transport is considered one of the most environmentally friendly means of transportation, still abrasion-related emissions enter the environment. One of the main sources of abrasion-related emissions are brakes. Nowadays, block brakes made of composite materials are mainly used in freight transport due to their improved noise characteristics. Older freight wagons have been converted from cast iron blocks to LL-blocks (low noise, low friction), whereas new freight wagons are equipped with K-blocks (composite). In passenger transportation brake pads consisting of composite materials are also used. Such composite brakes consist of a variety of organic and inorganic components. However, the composition of the individual brake pads is not regulated and therefore largely unknown. Due to the different kinds of brake pads and the variance in their chemical composition, there is currently very little information available on the composition of brake emissions from rail traffic.

In order to be able to assess the potential risk to humans and the environment, information on the chemical characteristics of the brake pads and the resulting emissions is required.

Therefore, the goal of the present study is to elucidate the chemical composition of different composite brake pads and their abrasions in test bench investigations. Thus, several analytical methods are used to characterize the compositions and identify tracer substances characteristic for brake emissions.

Identification of the inorganic components of the brake pads was conducted using X-ray fluorescence spectroscopy (XRF). For the different brake linings different compositions were obtained. Besides Fe as the main element, Al, Ba, Ca, K, S, Si, Ti, Zn and Zr, were detected in larger fractions depending on the brake pad. The polymeric compound could be identified as phenolic resin by pyrolysis gas

chromatography mass spectrometry (py-GC-MS). Moreover, eluates of the brake material and environmental samples taken along a railway track will be analyzed via inductively coupled plasma - mass spectrometry (ICP-MS) and liquid chromatography-high resolution mass spectrometry (LC-HRMS) to assess a possible entry into soil and water.

Overall, the collected data will be included into a future risk assessment and a cross-modal analysis of abrasion related emissions.

2.06.P-Th042 Challenges of Climate Change: Risk Mitigation of Plant Protection Products Containing Semi-Volatile Active Substances Using the Example of Clomazone

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Semi-volatile active substances represent a special challenge in authorisation of plant protection products. Their use leads to exposition of non-target areas not only through spray-drift, but also through volatilisation with subsequent deposition. The process of volatilisation is highly dependent on multiple factors, for example the formulation of the plant protection product, the application technique, and especially the weather conditions. Considering the ongoing global warming, the problems caused by the transport of active substances with a potential for volatilisation to non-target areas can therefore be expected to increase.

Standard risk mitigation measures like the use of drift-reducing equipment and no-spray buffer zones are frequently used to reduce the entry of plant protection products into adjacent areas via spray drift. The exposition of adjacent areas via volatilisation and deposition, however, can only in part be addressed by those measures. Clomazone is a prominent example of a semi-volatile active substance. Small amounts of clomazone can already lead to visible bleaching in non-target plants. In the past, cases of bleaching have mainly been limited to applications of plant protection products containing clomazone in late summer. More recently, even after applications in spring cases of bleaching have been observed. This may in part be explained by increased applications during that time of the year, but it might also reflect a tendency towards weather conditions that favor volatilisation due to climate change.

In the presentation, we describe the different factors that influence the volatilisation of clomazone, and the set of special risk mitigation measures that have been established in Germany for the use of clomazone-based plant protection products. These measures have effectively minimised the cases of bleaching of non-target plants. Also, we outline the possible consequences of climate change to the risk represented by semi-volatile active substances. Finally, we discuss to which extent mitigation measures might require an adjustment in case exposure of adjacent areas to semi-volatile substances increases.

2.06.P-Th043 In Vitro Human Alveolar Macrophage (ImmuPHAGE™) Responses to Particulate Matter Exposure

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Air pollution contributes to approximately 4.2 million deaths globally each year, with a significant portion attributed to respiratory diseases caused by particulate matter (PM). PM consists of microscopic particles that can penetrate the small airways, leading to oxidative stress and inflammation. Alveolar macrophages play a key role in both short- and long-term lung responses in the respiratory airways. Understanding their interaction with PM is essential for evaluating potential toxicological and immunological effects. This study investigates the in vitro respiratory toxicity of PM2.5 collected from Prishtina and Obiliq, two cities in Kosovo.

Human alveolar macrophages (ImmuPHAGETM) were exposed to PM2.5 (25, 50, 75, and 100µg/mL) for 24 and 48h. PrestoBlue reagent (Invitrogen, UK), CytoTox-ONE assay (Promega, UK) and fluorescent microspheres (ThermoFisher, UK) assessed average population responses for cell health and function. Individual cell responses using high-content image analysis (In Cell Analyzer 600, GE Healthcare, UK), were used to assess morphological features and cell health. Cells were fixed and treated with a cocktail of fluorescent dyes and individual cell phenotypes were profiled using four different morphology and cell stress descriptors as previously described.

Mitochondrial activity was significantly reduced ($p < 0.05$) for all exposure conditions assessed. Phagocytic activity was significantly reduced ($p < 0.05$) after 24h exposure but significantly increased after 48h exposure ($p < 0.05$). Individual cell profiling using high-content imaging revealed distinct phenotypic changes in alveolar macrophages exposed to PM identifying the evolution of macrophage response to PM whereby the initial response (24h) was associated with elevated cellular stress alone and

longer-term response (48h) was associated with enlarged cells and increased vacuolation. The combination of cell stress and morphological perturbations observed indicates a pathophysiological alveolar-macrophage response associated with adverse airway inflammation. This study demonstrates the value of in vitro tools in characterising individual cell responses to understanding the mechanism of lung response for environmental particulates. This approach can be used to assess potential acute and chronic impacts of PM in the airways to deepen our understanding of how particulate matter affects lung function and support the global effort to mitigate air pollution and protect public health.

2.06.P-Th045 The Impacts of Gaseous Air Pollution on Insect Fitness

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Air pollution is a major public health concern, contributing to approximately seven million deaths annually. While extensive research has focused on the human health impacts of air pollution, fewer studies have addressed its ecological consequences. Research on insects is particularly sparse, and they may be especially sensitive to air pollutants such as particulate matter (PM) and oxidising pollutants such as nitrogen dioxide (NO₂) and ground-level ozone (O₃). For example, flying insects can accumulate PM on their exoskeletons while foraging, and habitats exposed to diesel exhaust emissions tend to host significantly fewer insect pollinators. Most studies addressing this have pursued a field-based approach, and there is a notable lack of empirical evidence on how such pollutants directly affect insect fitness. This study fills this gap by assessing the impacts of NO₂ on the growth and fitness of *Spodoptera littoralis* (Lepidoptera: Noctuidae) using a specialised fumigation chamber system. By examining the effects of an environmentally relevant range of NO₂ concentrations, we quantify the risks to insect populations from direct exposure to air pollution. The results from this study provide crucial evidence about the effectiveness of current legislation to protect ecosystems from harmful pollutants.

2.06.P-Th046 The Kinetic, Modelling Study of Fenton-like with Organic Acids and Their Fate in the Aqueous Phase Fate

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The chemical element iron is ubiquitous in the aqueous phase of the Earth's atmosphere and plays a role in numerous chemical and photochemical processes. Moreover, it performs the function of a micronutrient for microorganisms, which in turn synthesise robust organic ligands. These ligands exert an influence on the solubility, reactivity and iron complex species, which could have an impact on the oxidation capacity in cloud water. Recent experimental observations indicate that in excess of 95% of the dissolved iron in clouds is complexed by organic ligands. The complexation of iron affects the reactivity of iron during the decomposition of hydrogen peroxide (H₂O₂), which is typically referred to as the 'Fenton-like' reaction. Furthermore, the Fenton-like reaction is utilised in water treatment, as it has the potential to effectively remove a range of organic pollutants.

Here we study the kinetic of the reaction of iron complexes of gluconic (GLU) and succinic acids (SUC) with H₂O₂ in respect to the conversion of Fe (II) at different peroxides and ligands concentrations in the aqueous phase. The investigation of Fe(II) to Fe(III) conversion was performed using the ortho-phenanthroline (oPhe) method also called the APHA 3500 colorimetric test and UV-VIS spectrometry. A kinetic model was developed using the COPASI software [6]. The Fenton-like reactions involving organic acids such as succinic and gluconic acids in the aqueous phase of clouds should be investigated in order to evaluate their influence to the iron catalytic cycles and hydroxyl radicals production in cloud water. Given the strong influence clouds have on the troposphere composition and subsequent impact on the climate. The second-order rate constant for the classical Fenton reaction in the absence of organic ligands at pH 5 was determined to be $k_{2nd} = 75 \pm 3 \text{ L mol}^{-1} \text{ s}^{-1}$. The apparent second-order rate constants for Fenton-like reactions were observed to increase in the presence of succinic and gluconic acids. The rate constant of reaction between H₂O₂ and Fe(II)-GLU complex in the presence of the organic ligand was determined to be $k_{2nd} = (2.2 \pm 0.8) \times 10^3 \text{ L mol}^{-1} \text{ s}^{-1}$ at pH = 5. A developed theoretical kinetic model was employed to provide an explanation of the observed results and to predict the fate of iron complexation under atmospheric conditions. The results obtained will contribute to a better understanding of the oxidation capacity by the Fenton-like reaction in clouds.

2.06.P-Th047 Determination of PAHs in Indoor House Dust in Barranquilla Colombia and Their Relationship with Health, Using the Biological Model *Caenorhabditis Elegans*

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Introduction: The presence of atmospheric pollutants including polycyclic aromatic hydrocarbons (PAHs),

inside homes, can affect the health and well-being of their occupants, becoming a risk factor for respiratory, cardiovascular, allergic disease, cancer and premature mortality among others. In Latin America, including Colombia, the impact on health of these substances present in indoor environments has been little studied.

Objective: The objective of this study was to determine the presence of PAHs in indoor dust from homes in the city of Barranquilla, Colombia, accumulated in the filters of air conditioners, and their possible effects on health using the biological model *Caenorhabditis elegans*.

Methodology: Dust inside the filters of air conditioners was collected from 29 residential dwellings in the 5 localities of the city of Barranquilla, from May to July 2024. The presence of PAHs in the dust was determined by gas chromatography-mass spectrometry (GC-MS). To assess the health risk of chronic exposure to PAHs from indoor dust, the biological model *C. elegans* was exposed for 48 h to different concentrations of aqueous extracts obtained from the dust. The criteria evaluated were lethality, fertility and locomotion in the wild-type strain N2 and oxidative stress expression in the *sod1* and *sod4* mutant transgenic strains. Data are expressed as mean $\pm$ SEM. ANOVA was used for statistical analysis.

GraphPad Prism was used, and $p < 0.05$ was considered statistically significant.

Results: The data obtained provided evidence for the presence of PAHs in varying concentrations in indoor dust from homes in the city of Barranquilla. Exposure to dust extracts affected physiological parameters in *C. elegans*. Oxidative stress-related gene expression in GFP transgenic nematodes was related to the presence of PAHs..

Conclusions: The data obtained in this research can be used as a basis for future research to accompany governmental decisions aimed at regulating the generation of pollutants that negatively affect the quality of air breathed in homes.

2.06.P-Th049 Incorporation of Environmental Airborne Nanoparticles in Human Lung Cells

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The present study demonstrates the cellular internalisation of environmental settleable atmospheric particulate matter (SePM) and PM10 in bronchial epithelial and fibroblast human lung cells taken in urban areas subject to the major influences of the steel industry, iron pelletizing and civil construction. High resolution transmission electron microscopy (HRTEM), nanocrystallography, electron energy loss spectroscopy (EELS), nanoscale secondary ion mass spectrometry (NanoSIMS) and inductively coupled plasma mass Spectrometry (ICP-MS) were applied to characterise the SePM, including nanoparticles, at the subcellular level. PM10 shows higher element uptake in comparison to SePM, in which the metallurgical sources were more incorporated than those from civil construction/vehicular sources. Fibroblasts showed a higher metal incorporation than epithelial cells after SePM exposure. The internalisation of Al, Si, Ti, Fe and Zr was observed, with nanoparticles free in the nucleus (membrane not identified) and membrane-bound particles/clusters in the cell cytoplasm. Chemical and ultrastructural analyses demonstrate an association of titanium, iron and zirconium nanoparticles with metallurgical sources and secondly with vehicular emissions. This study raises concerns about releases of PM from metalliferous areas, requiring stricter environmental regulations of atmospheric quality to protect human health.

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2.07 Marine Ecotoxicology: Anthropogenic Pressures on Marine Organisms and Habitats, Current Challenges and Solutions

2.07.T-01 Intraspecific Differentiation and Importance of Cold Gametogenesis for Heat Tolerance in the Golden Kelp *Laminaria ochroleuca*

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Climate change is driving shifts in species distributions and adaptations to new environmental conditions.

Since sessile species cannot escape stressors, thermal plasticity becomes critical for survival in changing environments, as it allows organisms to adapt. Kelps (Laminariales, Phaeophyceae) are sessile and important "ecosystem engineers", providing essential ecosystem services and supporting biodiversity. This research investigates whether the optimal thermal reproductive range of *Laminaria ochroleuca*, a warm-temperate kelp species distributed from southern England to Morocco, including the Strait of Messina and the Azores, varies among populations and whether gametogenesis and sporophyte development temperatures affect the thermal tolerance and growth of F1 sporophytes.

Gametophytes from four populations representative of the species distribution (France, Morocco, Italy, Portugal) were exposed to temperatures allowing gametophyte reproduction (11°C, 13°C, 16°C, 18°C) under controlled conditions to assess reproductive success. Additionally, thermal tolerance of F1 sporophytes from one population was tested following gametogenesis at 11°C and 16°C and sporophyte growth rates and survival were assessed after exposure to sub-lethal and lethal temperatures.

Results showed intraspecific ecotype differentiation. The Mediterranean population had higher reproductive success at the highest temperature tested (18°C), reflecting adaptation to the region's rising sea temperatures. In contrast, Atlantic populations performed better at lower temperatures (11°C, 16°C), consistent with local oceanographic conditions, that include cooler seawater temperatures and upwelling events. Additionally, F1 sporophytes developed at a cooler temperature (11°C) exhibited enhanced survival under heat stress (23°C), suggesting the presence of trans-generational plasticity and carry-over effects.

These findings highlight the importance of phenotypic plasticity in enabling local adaptation and suggest vulnerabilities to climate change. Rising ocean temperatures could lead to the loss of southern populations in North Atlantic, though deep refugia like the Messina Strait may persist. The results also show the possibility of developing heat-tolerant strains to support kelp restoration and aquaculture. By advancing understanding of *L. ochroleuca* thermal responses, this research provides valuable insights into managing and conserving kelp forests in a warming world.

2.07.T-02 Monitoring Plastic Additives and Micro- and Nanoplastics Contamination in Ireland's Coastal Regions

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Marine pollution caused by micro- and nanoplastics (MNPLs) and their associated additives presents increasingly complex ecological and toxicological challenges. These pollutants impact individual organisms and disrupt marine ecosystems by introducing bioaccumulative chemical loads through food chains. Plastic additives released during the degradation of plastics can affect endocrine functions, reproduction, and organism resilience to environmental stressors. Research on the Irish Sea is particularly scarce, especially in coastal areas. This region's biodiversity, economic reliance on marine resources, and exposure to Atlantic currents make it critical to assess the presence and impact of MNPLs and plastic additives in these waters. Water and sediment samples were collected from 10 sites across the Irish Sea. For water, two liters per sampling point were processed via solid-phase extraction (SPE) for additives and filtration for MNPLs, followed by ultrasonic-assisted extraction (USAE) using toluene. Sediments were analyzed by weighing five grams per sample and subjecting them to USAE with methanol for additives and toluene for MNPLs. Plastic additives were analyzed using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS), employing a reverse-phase column. MNPLs were identified through size exclusion liquid chromatography coupled to mass spectrometry (LC-SEC-HRMS).

The analysis revealed a wide range of plastic additives, including plasticizers such as phthalates and adipates, flame retardants, and protective agents like antioxidants and corrosion inhibitors. Flame retardants had the highest concentrations in sediment samples (0.05–100 ng·g⁻¹), while corrosion inhibitors were found in lower ranges (1–15 ng·g⁻¹). Sites near ports and river mouths exhibited the highest pollution levels compared to seemingly isolated beaches.

Regarding MNPLs, common polymers such as polyethylene, polyamide, and polyvinylidene fluoride (PVF) were identified. Polymer concentrations in water ranged from 0.086 to 0.94 µg·L⁻¹, while polyamide was detected at lower concentrations (0.27–134 ng·L⁻¹). These polymers originate from diverse sources, including food packaging, synthetic rubber products, cosmetics, and electronic components. These findings highlight the environmental persistence and bioaccumulative nature of MNPLs and their additives, posing risks to marine life and human health through food web contamination.

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2.07.T-03 Mapping the Fate, Ecological Interactions and Impacts of Microplastic Pollution Across the Galapagos Marine Reserve

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Plastic leakage from the Eastern Pacific rim countries of Ecuador and Peru is captured by the South Pacific Subtropical Gyre, transporting it towards the open ocean, where it pollutes oceanic islands such as Galápagos, famous for its unique biodiversity and a global conservation priority. Here we report the findings of our 5-year collaborative programme to characterise the widespread plastic contamination of the Galapagos marine ecosystem and determine how particle interactions with the environmental conditions and ecological processes of this unique archipelago impact microplastic fate and behaviour and uptake into wildlife. Field sampling campaigns focussing on the island of San Cristobal, the Eastern-most Island of Galapagos most exposed to the Humbolt current, were undertaken from 2018-2023. Samples were collected around the island for the sea surface, benthic sediments, beaches, mangroves and representative invertebrate biota for microplastics in combination with beach and mangrove macroplastic surveys. Our data reveals widespread and highly variable microplastic contamination of San Cristobal of both benthic sediments (6.7 86.7 particles·kg⁻¹) and surface seawater (0.61 10.81 particles·m⁻³). Grab sampling (whole water 1 litre samples filtered to 1.2 µm) revealed elevated concentrations of up to 23.3 particles per litre (equivalent to 23,333 particles per m⁻³) at the town s outflow pipe, mostly comprising anthropogenic cellulose fibres. Mangroves were found to accumulate both macroplastic and microplastics, with concentrations of microplastics found in seawater within the mangroves found to be 4 orders of magnitude higher than that in their surrounding coastal waters. We have found evidence of ingestion of microplastics within every species investigated to date, from sea urchins to the iconic Galapagos marine iguanas. Our results highlight different sources of plastic contamination for different areas of the Galapagos archipelago, and their interactions with complex ecosystems. These factors act to influence hot spots of plastic accumulation and determine uptake rates into Galapagos wildlife. Working closely with the Galapagos National Park, our data is now supporting local management decisions, mitigation actions and clean up efforts to reduce plastic pollution in Galapagos.

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2.07.T-04 Maternal Transfer of PFAS in Marine Mammals via Lactation: Quantitative and Non-Targeted Analyses

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Per- and polyfluoroalkyl substances (PFAS) are a class of anthropogenic chemicals widely used in a variety of consumer and industrial applications dating back to the 1950s. Due to their carbon-fluorine bonds, PFAS are resistant to thermal and chemical degradation and readily enter the environment, thus being ubiquitous in all ecosystems. Moreover, PFAS bioaccumulate, biomagnify, and have significant impacts on mammalian health. These impacts are exemplified in neonates during crucial stages of development, leading to changes in adiposity, decreased immune response, and disrupted thyroid function. High neonatal body burden of PFAS is greatly attributed to lactational transfer. Marine mammals are regarded as critical sentinel species for persistent organic pollutants (POPs) in aquatic ecosystems. However, there is little data on how PFAS perturb marine mammalian health, and it is currently unclear which PFAS analytes are transferred via lactation and how such transfer affects the biological functions of young mammals. For species that rely on milk as their main food source during key growth and developmental milestones, high milk PFAS concentrations could pose a serious threat to future ecological fitness with greater ramifications to trophic networks. Therefore, milk is a vital yet underutilized matrix for evaluating neonatal exposure despite its potential value in providing critical toxicologic data. This work presents an optimized extraction method for non-targeted analyses (NTA) of PFAS in mammalian milk, developed using Quick Easy Cheap Effective Rugged and Safe (QuEChERS) salts and dispersive solid-phase extraction (dSPE) followed by NTA with a platform coupling liquid chromatography, ion mobility spectrometry, and mass spectrometry (LC-IMS-MS). This optimized extraction showcased high recovery across four PFAS classes compared to alternative clean-up methods. The extraction method was also further optimized for smaller sample volumes while maintaining the ability to detect low PFAS concentrations (<1 ng/mL). These optimized methods will be applied to a host of longitudinal marine mammal milk samples for quantitative analysis and NTA, including apex marine predators, like bottlenose dolphins and sea lions. In addition to quantification of PFAS to track trends in concentration, NTA will be

applied to identify unique PFAS species that can inform future toxicologic studies with the potential to identify novel routes of exposure.

2.07.T-05 Temporal Variations of Marine Toxins in Catalan (NE Spain) Seawater: A Suspect Screening Comparison

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Marine biotoxins, produced by microalgae, pose a growing threat to marine ecosystems, fisheries, and human health. These secondary metabolites can accumulate in marine organisms and enter the food chain, potentially causing severe intoxication in humans. This issue is particularly relevant in the Mediterranean Sea, where environmental changes and anthropogenic activities influence the occurrence and distribution of biotoxins. This study evaluates the temporal variation of marine biotoxins in surface seawater from the Catalan coast (NE Spain), comparing results from samples taken along 2024 and with a retrospective evaluation of samples previously studied by our group a decade ago to assess potential shifts in toxin diversity and concentrations.

A total of 36 seawater samples from the Catalan coast (NE Spain) were collected and analyzed using ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry. The results showed that okadaic acid (OA), a lipophilic toxin, was identified as the most prevalent compound, detected in 88% of the samples with an average concentration of 730 ng/L. The highest OA levels were observed in areas heavily influenced by urbanization, agriculture, or industrial activities, highlighting the role of anthropogenic inputs in enhancing eutrophication and microalgae growth. Beyond OA, the retrospective analysis revealed additional marine biotoxins, including 20-methyl spirolide G, azaspiracids (AZA-1, AZA-2, and AZA-3), pectenotoxin-1, and verrucarol. This marks the first identification of several of these compounds in seawater samples from the Catalan coast (NE Spain). Comparing the results of the lipophilic toxins with the samples taken along 2024, no lipophilic marine toxins were detected in the sampling sites.

This study provides valuable insights into the presence and distribution of marine biotoxins along the Catalan coast (NE Spain), highlighting the importance of continuous monitoring in understanding their dynamics. The absence of detectable lipophilic marine toxins in the first suspect screening of 2024 emphasizes the seasonal variability of these compounds and the necessity to further evaluate the lipophilic fraction as well as to monitor marine toxins along seasonal variations.

2.07.P Marine Ecotoxicology: Anthropogenic Pressures on Marine Organisms and Habitats, Current Challenges and Solutions

2.07.P-Th050 Effects of Short- and Long-Term Exposure to Natural Acidified Conditions in the Limpet *Patella Caerulea*

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Ocean acidification (OA) entails a detrimental impact on calcifying organisms by reducing carbonate availability, which makes their calcareous structures more fragile. Nevertheless, the gastropod *Patella caerulea* is able to inhabit the Castello Aragonese vent systems, a site naturally acidified by the volcanic CO₂ emitted from the seafloor. This could happen through plasticity and/or adaptive mechanisms which still mostly remain unknown. The aim of this study is to compare the long-term strategies adopted by limpets that spent their entire life cycle in naturally acidified conditions with the short-term ones induced by an exposure of limpets to OA through an in situ transplant experiment.

In order to evaluate the effects of long-term exposure to OA, specimens of *P. caerulea* were collected from the pH gradient (N1: pH = 8.1; N2: pH = 7.7; N3: pH = < 7.4) of the vent and from the ambient pH site San Pietro (SP: pH = 8.1). For the transplant experiment, specimens of *P. caerulea* were collected from an ambient pH site (pH = 8.1) and transplanted to the three different stations of the vent for 30 days during the summer season. In both cases, molecular, biochemical, physiological, and metabolic mechanisms were investigated.

The most relevant result concerning physiology is a significant increase in respiration rate and a decrease in ammonia excretion rate in limpets collected in N3 only during summer season. Conversely, no effects were observed in organisms from the transplant experiment. Regarding energy-related metabolism, a

common trend was observed in both short- and long-term exposure, with a significant increase in glycogen content in limpets collected from the acidified sites of the vent. Moreover, OA induced oxidative stress only in transplanted limpets.

Overall, our results suggest that multiple mechanisms may contribute to boost tolerance to OA in the limpet *P. caerulea*, including ecological interactions, physiological functions, and energetic performance. The short-term exposure to OA didn't affect the survival of limpets transplanted to acidified conditions, which may be due to compensatory mechanisms or to the short duration of the experiment. Therefore, to gain a comprehensive understanding of the effects of OA, it is essential to examine adaptive strategies that may be developed over multiple generations, besides physiological adjustments induced by short-term exposure to low pH conditions.

2.07.P-Th051 Can Oxygen Supersaturation Boost Thermal Tolerance in Marine Fauna?

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Marine coastal environments are significantly impacted by the phenomenon of global warming. Abrupt temperature increases, like marine heatwaves, can affect animal physiology, abundance, distribution, trophic interactions, survival, and mortality. These negative effects are often associated with an increased aerobic metabolic demand resulting from the thermal stress. However, coastal environments are typically characterized by the presence of photosynthetic organisms that serve as primary producers and may help to mitigate this trend by increasing the oxygen availability. In this experiment, oxygen supersaturation was used to simulate conditions of high primary production and was tested in combination with thermal stress to assess its impact on animal physiology and survival of some taxa. Species from 2 different phyla (Echinoderms and Cnidarians) were chosen among the most common species found in the Venice lagoon. Organisms were collected during summer 2024, from three different sites characterized by large coverage of macrophytes and different exposure to anthropogenic impacts. Lethal and sublethal site effects were tested on the species under different conditions i.e., by exposing the individuals to a thermal slope (1°C every 30 minutes) under both normal (~90% of dissolved oxygen) and supersaturated (~160% of dissolved oxygen, mean daytime value of oxygen due to primary production) oxygen levels. The results indicate that oxygen supersaturation can increase the thermal tolerance of the tested animals, both extending survival and reducing the metabolic rate under heatwaves. No significant differences were detected among sites, demonstrating the ubiquity of the dynamic tested herein. These results shed light on an understudied intrinsic interaction in marine ecosystems, providing fundamental knowledge about the resilience of organisms to global warming within the context of their natural environment.

2.07.P-Th053 Physiological Responses and Adaptive Mechanisms of *Ulva* sp. to Marine Heatwaves

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Marine heatwaves (MHWs) are becoming more frequent and intense due to climate change, significantly impacting marine ecosystems, including macroalgae such as *Ulva* spp.

This study aims to investigate the physiological responses of *Ulva* sp. to simulated heatwave conditions and to evaluate potential adaptive mechanisms when exposed to multiple heatwaves.

Algal samples were subjected to three thermal regimes: a single heatwave (SH), two consecutive heatwaves (DH), and a control condition without thermal stress.

The heatwave simulation involved raising the water temperature from 18°C to 24°C for 7 days, followed by a 7-day recovery period at 18°C. Physiological changes were assessed by measuring biomolecule levels (pigments, proteins, sugars, and starch) and photosynthetic performance using photosynthesis-irradiance (PI) curves at the end of each heatwave peak and during each subsequent recovery phase.

The analysis of photosynthetic parameters and biomolecule levels revealed distinct physiological responses to thermal stress. Both the SH and DH treatments showed a significant increase in photosynthetic activity (P_{max}) during heatwave peaks and recovery periods, although the response varied between treatments. The DH treatment exhibited a higher P_{max} during the initial recovery phase and at the second heatwave peak, suggesting potential adaptive responses such as increased synthesis of photosynthetic pigments and enhanced energy allocation for cellular repair. Additionally, the rise in chlorophyll-a and carotenoid levels in the DH treatment indicated that *Ulva* sp. might use pigments to improve light capture and provide antioxidant protection under stress. Protein levels also increased in the DH treatment, suggesting the synthesis of structural and stress-response proteins that help maintain cellular integrity during repeated heat exposure.

Overall, *Ulva* sp. demonstrated physiological plasticity in response to thermal stress, indicating a potential resilience to future warming events.

The results provide valuable insights into the adaptive capacity of macroalgae, with significant implications for both the conservation of marine ecosystems facing climate-induced thermal stress and the rapidly growing industry of macroalgae cultivation for food and pharmaceutical purposes.

2.07.P-Th054 Health and Reproduction of the Red Seaweed *Palmaria palmata* in a Climate Change Scenario

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In cold and temperate climates, macroalgae constitute the main habitat-forming species for coastal ecosystems and provide various benefits to local human communities. *Palmaria palmata* is an ecologically relevant seaweed distributed throughout the North Atlantic. It is also a species of interest for the development of at-sea aquaculture for human consumption due to attractive nutritional and bioactive properties. It is, however, characterised by a complex life cycle requiring different life stages (sporophytes, male and female gametophytes) to reproduce in a short, specific sequence.

Climate change is increasing the frequency and intensity of extreme weather events, including marine heatwaves (MHW) and heavy rainfalls, both leading to short-term but intense changes in environmental conditions. Additionally, industrial activities emit increasing quantities and variety of pollutants, many of which eventually end up in aquatic systems. The effects of these emerging climatic and anthropogenic pressures on seaweed populations are poorly documented, leading to inadequate or inexistent conservation and protection policies.

In this research, we investigated the individual and synergetic effects of MHW, intense rainfalls and pollutants on the health and reproductive ability of *P. palmata*. Unique (3°C, 7 days) and repeated (3°C, 3 x 7-days cycles) temperature increases, and salinity decreases (4-10 PSU, 14 days) were used to simulate MHW and intense rainfalls, respectively. Effluent from a local wastewater treatment plant was diluted to three environmentally relevant concentrations to represent pollutants routinely present in marine systems. Seaweed health was monitored before, during and for 10 weeks after exposures by measuring growth, photosynthetic activity (maximum quantum yield and electron transport rate), oxidative stress (lipid peroxidation) and chemical composition (pigments, proteins, polysaccharides and phenolic compounds). All plants were placed in conditions favouring sporogenesis to monitor effects of treatments on reproductive ability, and spore output was quantified to measure reproductive success.

The identification and quantification of changes in wild seaweed populations, which influence their life cycle dynamics and overall health status, provided an effective method for assessing the impacts of climate change on ecosystems.

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2.07.P-Th055 Evaluating the Potential of Chemical and Thermal Priming to Enhance Thermal Tolerance in Juvenile Sporophytes of the Golden Kelp *Laminaria ochroleuca*

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Laminaria ochroleuca is increasingly threatened by rising seawater temperatures due to climate change, with critical ecological and economic implications. This study investigated whether chemical and thermal priming could enhance the thermal tolerance of juvenile sporophytes. While priming techniques in terrestrial plants have been extensively studied, their application in seaweeds remains unexplored. This research aimed to assess their potential for improving kelp resilience, supporting restoration and aquaculture.

Juvenile sporophytes of *L. ochroleuca* were exposed to four priming treatments over 2 days: control (13°C), thermal priming (23°C), chemical priming with 0.05 mM hydrogen peroxide (H₂O₂) at 13°C, and thermo-chemical priming (0.05 mM H₂O₂ at 23°C). Sporophytes were then subjected to temperatures including sub-lethal and lethal for 14 days. At the start and end of the experiment, growth rates were evaluated by sporophyte area, and photosynthetic performance was assessed using chlorophyll fluorescence parameters (Fv/Fm and rapid light curves).

Results revealed that priming treatments had limited effects on thermal tolerance. Growth rates gradually

declined as temperature increased, and 25°C proved lethal, resulting in severe stress or mortality. Photosynthetic performance similarly decreased at higher temperatures, likely due to heat-induced photoinhibition. At 13°C, priming treatments improved some photosynthetic parameters, suggesting enhanced photosynthetic activity under optimal conditions. However, these benefits did not seem to persist under heat stress.

The lack of significant effects may reflect the short duration of the priming treatments or the absence of lasting stress memory in juvenile sporophytes. While priming did not enhance heat tolerance in this study, it may be more effective when applied to earlier life stages, such as gametophytes, where transgenerational plasticity and carry-over effects could improve resilience in offspring. Future research should investigate whether longer priming exposure enhances heat tolerance in sporophytes and explore the role of epigenetic modifications in stress tolerance. This study highlights the challenges of applying priming techniques to kelps, the need to understand phenotypic plasticity in macroalgae and to develop innovative approaches to address climate change. Developing stress-tolerant strains through priming could benefit both mariculture industry and kelp forest restoration.

2.07.P-Th056 Mauritius Oil Spill: Hydrocarbon Residues Remain in the Mangrove Systems Three Years Later

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The grounding of the MV Wakashio off the coast of Mauritius in July 2020 was the world's first major spillage of Very Low Sulfur Fuel Oil (VLSFO). Nearly 3 years following the spill, sediments were collected from two mangroves ecosystems: a reference site and an impacted site, with sediments analysed by comprehensive two-dimensional gas chromatography and by gas chromatography-mass spectrometry. No petroleum hydrocarbons were identified at the reference site. At the impacted site, molecular biomarkers matched the profile of the MV Wakashio VLSFO. A linear regression of terpenoid ratios between the original Wakashio fuel oil and that of the sediments collected at the impacted site produced a R^2 of 0.9995, indicating a positive match between them. It was observed that under the Mauritius tropical conditions, the VLSFO has undergone significant weathering with several mono- and polycyclic aromatic hydrocarbons being absent from the hydrocarbon mixture. However, some environmentally resilient 4- and 5-rings PAHs were still present in the sediments at the impacted site. Overall, most of the compounds known to be toxic to marine organisms have dissipated but the continued presence of oil in the mangrove systems is known to negatively impact the mangrove forests in the long term.

2.07.P-Th058 Influence of Sun Creams on Corals

Guido Gonsior, Maren Dill, Sara Cuellar and Gundula Gonsior, GG BioTech Design, Germany

Corals play a crucial role in the marine ecosystem. They form one of the most species-rich ecosystems on earth. And coral reefs are one of the most endangered ecosystems. The harmfulness to coral ecosystems of various UV filters in sun creams, which are supposed to protect our skin from UV light, was investigated and appears to have been proven. However, the current state of the data is not yet clear. Above all, the question remains as to whether the environmentally relevant concentrations are sufficient to cause toxic effects. In addition, there are still no standardized lab tests. Further, the focus here has often been on individual components of sun creams only. But there are many different formulations on the market. These are not only worn in the water when swimming. Exposure via wastewater after showers should be viewed critically, too. Especially as many hotels located on tropical seas have inadequate wastewater treatment systems. We present data from various sun creams that were applied directly into the water or discharged via wastewater.

2.07.P-Th059 Toxicity of Arsenate and Arsenite to Coral Species *Acropora cervicornis* and *Orbicella faveolata*

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Port Everglades is a large seaport in Fort Lauderdale, Florida, located adjacent to the coral communities at the northernmost end of the Florida Reef Tract. There are questions regarding how resuspended sediments from the planned dredging and expansion of the port could impact nearby coral reefs. Numerical sediment quality assessment guidelines as well as threshold effect levels (TELs) and probable effects levels (PELs) have been derived for nine metals in Florida coastal waters. A recent study found concentrations of arsenic

above TEL (7.24 µg/g) and PEL (41.6 µg/g) values in the port s sediments. Arsenic enters coastal waterways through anthropogenic activities and natural sedimentary processes. In corals, exposure to arsenic-contaminated sediments or solubilized arsenic may disrupt vital biological functions and symbiotic relationships, leading to coral bleaching and mortality. To assess the possible effects of soluble arsenic exposure on adjacent reef systems, the acute toxicity of arsenic species arsenate (As(V)) and arsenite (As(III)) were tested with coral species *Acropora cervicornis* and *Orbicella faveolata* using 96-hour static renewal assays. Each exposure assay included six treatments, consisting of five concentrations (ranging from 0.06-5 mg/L for As(V) and 0.03-2.5 mg/L for As(III)) and a negative control, with six replicate beakers per treatment. Effects were evaluated based on coral mortality (LC50), coral condition (EC50), and the photosynthetic efficiency of the dinoflagellate symbiont (IC50). Arsenic concentrations were analytically verified. During the exposure assays, arsenate and arsenite were found to induce polyp discoloration and polyp retraction. The three highest concentrations of As(V) solutions resulted in 100% mortality after a 24-hour period, while the highest concentration of As(III) led to 100% mortality after 48 hours. Arsenite, despite being tested at lower concentrations and within a narrower range, exhibited greater toxicity than arsenate. This observation is consistent with prior research conducted on other marine organisms. The results of these experiments provide new data to support management decisions relating to the testing and disposal of arsenic-contaminated sediments in tropical coastal environments.

Disclaimer/Disclosure: Experimental corals were collected and retained under Florida Fish and Wildlife Conservation Commission Special Activity License SAL-24-2609-SRP.

2.07.P-Th060 Coral Reef Research: Identification of Chemical Stressors with Negative Influence on Tropic Costal Ecosystems

Guido Gonsior, Gundula Gonsior, Sara Cuellar and Maren Dill, GG BioTech Design, Germany

Coral reefs act as natural barriers that protect coasts from erosion and storm damage and reefs provide an important source of food through fisheries and other marine resources. Beside these corals also contribute to carbon sequestration by absorbing CO₂ from the atmosphere and storing it in the form of limestone. Also, tourism based on coral reefs is an important source of income for many countries.

Overall, coral reefs are essential to the health of the oceans and the well-being of the people who depend on them. The protection and conservation of these ecosystems is therefore of paramount importance. The deterioration of water quality in coastal waters is jeopardising marine fauna. The highly sensitive corals in particular are under increasing pressure from industrial wastewater, fertilisers and pesticides from agriculture, as well as from cosmetics.

However, up to now, less focus was given on the pharmaceutical which were identified to have a huge effect on ecosystems. Therefore, first studies were initiated to clarify potential issues. We present data of effects from selected pharmaceuticals (e.g., Valsartan, Diclofenac) on coral eco systems.

2.07.P-Th061 Dietary Markers and Contaminant Trends in Eastern Beaufort Sea Belugas: Exploring the Impact of Prey Shifts on PCBs and PBDEs

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The Eastern Beaufort Sea (EBS) beluga is one of Canada s largest populations and is ethically harvested by the Inuvialuit communities in the Northwest Territories. Climate change in the Arctic has been linked to a potential shift in diet from mainly Arctic Cod to Capelin, which may also result in a decline in body condition of belugas, especially for juvenile males and females. However, how this climate-induced prey shift may impact the animal s exposure to environmental contaminants remains unclear. Given recent observations of changes in the diet of EBS belugas, this study aimed to explore the relationships between the diet (as estimated by dietary fatty acid (FA) markers) and concentrations of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in their blubber over 22 years. Specific objectives were to evaluate temporal trends of these contaminants as well as their relationships with specific FA dietary markers (20:1n-7, 20:1n-9, 20:4n-6, 20:5n-3, 22:1n-11 and 22:6n-3) and principal components derived from FA signatures. Our results highlighted no significant change in blubber PCB and PBDE concentrations between 1995 and 2017. However, concentrations of both PCBs and PBDEs peaked in blubber samples in 2014, coinciding with a low Arctic Cod biomass and the lowest recorded body condition of belugas during the same year. Moreover, several monounsaturated FAs (20:1n-7, 20:1n-9, 22:1n-11) were positively correlated with PCB concentrations, whereas specific polyunsaturated FAs (20:4n-6 and 22:6n-3) were negatively correlated. We also observed that principal components derived

from FA signatures were correlated with blubber PCB concentrations. PBDE data are currently undergoing analysis, but we predict similar relationships with FA markers as this class of contaminants exhibits comparable physico-chemical properties. These results suggest that dietary markers may be valuable predictors of PCB and potentially PBDE exposure in EBS belugas. Our observations also hint towards a complex connection between diet shifts, decreasing body condition and contaminant exposure in EBS belugas and underscores the necessity for further research and monitoring of contaminants in Arctic marine mammals.

2.07.P-Th063 Comparison of Metallic Concentrations in Two Breeding Colonies of the Endangered African Penguin (*Spheniscus demersus*)

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The African penguin, *Spheniscus demersus*, is endemic to the southern African coastline. The IUCN (International Union for Conservation of Nature) has recently listed them from Endangered to Critically Endangered due to their significant decrease in numbers and their continual shifting of main breeding concentrations. Their decline is attributed to factors such as low fishing stock through overfishing, oil pollution, climate change, and displacement from previous breeding areas through human and predator disturbance. In this study, we examine metal pollution as a potential contributor to the decline of *S. demersus*. This was achieved by determining whether their egg content and eggshells could be used as indicators of metal pollution. We collected eggs from Bird Island and Robben Island and measured 20 metallic elements in egg content and eggshells separately.

2.07.P-Th064 Tracing History of Microplastic Pollution and Associated Environmental Changes by Using Fjord Sediment Archives

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Plastic particles pose a significant threat to the marine environment. Plastics will eventually degrade, breaking down and creating smaller-sized particles, so-called microplastics. In the ocean, sooner or later microplastics will sink and end up on the sea floor. Therefore, marine sediments are an important reservoir for microplastic (MP) accumulation. By combining radiometric dating of sediment cores with the extraction of MPs from different core intervals representing different time periods, the rate of plastic accumulation over time can be determined.

In this study, we collected sediment cores in the Hakefjord, in proximity to Stenungsund, a small town on the Swedish west coast and home to Sweden's largest producer of plastic pellets. There are two manufactures: one is producing polyethylene (PE) and the other one is producing polyvinyl chloride (PVC) pellets. The production of PE started in 1963 and had a constant production increase since then. Today approximately 0.75 million tonnes of PE pellets are produced yearly, which corresponds to 5% of the European market. The PVC industry has been in the area since 1968, and now they produce around 0.23 million tonnes of PVC pellets yearly.

Based on the radiometric dating by ²¹⁰Pb, ¹³⁷Cs and ²²⁶Ra, the sediment intervals taken in 2cm resolution are pooled according to their age. Particles are separated and sorted into size classes; >300 µm, 100-300 µm, and 30-100 µm. Particles are identified with Fourier-transform infrared spectroscopy (FTIR) and Raman microscopy. By linking amount of MPs in the sediment to temporal industrial development, it is possible to track the emission of plastic pellets since the production started. Production volume and allowed emission rates have changed over the years and we aim to define the link between the two by using sediment archives.

Linked to the development of plastic industries in the fjord, we are also studying potential changes in benthic biodiversity by looking at meiofauna (metazoans and foraminifera) in the sediment cores by using conventional morphological methods and environmental DNA (eDNA). eDNA refers to whatever genetic material that can be recovered from an environmental sample. The species composition is estimated by metabarcoding based on molecular markers. In foraminiferal analysis, we study potential incorporation of small micro- and nanoplastics in the shell matrix by using SEM-Raman and how it changed over time.

2.07.P-Th065 The Impacts of Microplastic Ingestion on Zooplankton Lipid Physiology and Ecosystem Functioning: A Case Study on Arctic Copepods

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The accumulation of plastic waste in marine environments, particularly microplastics (MPs), presents a critical environmental challenge. MPs are pervasive across marine ecosystems, from surface waters to deep-sea sediments. Extensive research has highlighted their hazardous nature due to their bioavailability, capacity to vector pathogens, and potential to adsorb and transfer toxic substances. Zooplankton, as a pivotal component of aquatic ecosystems, is particularly vulnerable to MPs, which readily integrate into marine trophic chains, posing ecological risks through ingestion, bioaccumulation, and trophic transfer. These risks are particularly acute in Arctic marine ecosystems, where heightened vulnerability to climate change and other stressors amplifies their impact on biota.

Among arctic zooplankton, copepods are of particular interest due to their crucial role in marine biogeochemical processes, such as carbon sequestration. One of the mechanisms in this process is the lipid pump, where copepods accumulate energy-rich lipids during their active feeding seasons and subsequently transport these lipids to deeper ocean layers during overwintering and diapause. Despite their importance, the potential effects of MPs ingestion on the lipid metabolism of Arctic copepods remain underexplored. Meanwhile, feeding experiments with MPs can provide critical insights into how their ingestion may disrupt lipid metabolism, which is essential for energy storage in these ecologically significant copepod species.

This study investigated the effects of MPs ingestion on Arctic copepods *Calanus hyperboreus* during feeding experiments, where specimens were exposed to varying concentrations of MPs under conditions of food availability and deprivation. The study examined changes in lipid metabolism and elemental composition (carbon and nitrogen content, along with isotopic signatures) as indicators of nutritional stress and physiological disruption. By analyzing shifts in lipid profiles of copepods along with their carbon and nitrogen content across different treatment groups, this research provides insights into how MPs ingestion affects the nutritional quality and energy storage of this key Arctic species.

The results of this study are expected to elucidate the physiological impacts of MPs ingestion on Arctic copepods and their broader ecological consequences of the Arctic marine ecosystems carbon sequestration processes.

2.07.P-Th066 Effects of Ammonia and Methanol Spills on Zooplankton in East Baltic and Bothnian Bay

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The shipping industry is shifting to greener fuels, like ammonia (NH₃) and methanol (MeOH), following the goals of the International Maritime Organization (IMO) to substantially reduce greenhouse gas (GHG) emissions. This shift imposes the risk of spilling these substances into the marine environment with potential adverse effects on marine food webs. However, the lethal and sub-lethal effects, threshold concentrations or cumulative effects with other stressors on plankton communities are not known, not even in highly trafficked areas like the Baltic and North Seas. Given the significance of zooplankton in energy transfer within marine food webs, it is crucial to understand their response to NH₃ and MeOH spills under different environmental conditions.

In-situ experiments were performed in East Baltic and Bothnian Sea, during the COMBINE 3 scientific cruise on R/V Aranda. NH₃ effects were tested on seven zooplankton species under 24h. *Temora longicornis*, *Eurytemora affinis*, *Limnocalanus macrurus*, *Thermocyclops* spp., *Acartia bifilosa*, *Bosmina longispina*, and *daphnia cucullata*. Mortality was the endpoint of the experiments, and 5 concentrations of the chemicals were tested in each experiment. Measurements of carapace and total length was taken from all subjects. Moreover, the fecal pellet production was measured for all copepod species, and females carrying eggs of *E. affinis* were isolated in well plates for hatching success. Data was analyzed with the R statistical software and dose-response curves were fitted to the data.

Our preliminary results on the environmental conditions show a distinction between the northern Bothnian bay and the east Baltic water. LC50 for ammonia was between 0.5 and 1.5 for all species but *Bosmina* spp. which had low mortality in all tested concentrations. All copepods were negatively affected. Fecal pellet production was affected in all copepods but cyclopoids. The hatching success of *E. affinis* was significantly affected by the tested concentrations.

Our results indicate that potential spills of ammonia and methanol will affect the examined ecosystems in areas where concentrations reach the tested concentrations. The risk of spills will increase as more ships shifting to Ammonia and Methanol fuels. Moreover, our study suggests that risk assessments should be performed in situ, as different environmental parameters might affect the outcome of Ammonia or Methanol loading in marine ecosystems.

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2.07.P-Th068 Toxicological Assessment and Environmental Distribution of Tetracene Along the Yellow Sea Coast

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The aryl hydrocarbon receptor (AhR) agonist, tetracene is a highly toxic compound with notable environmental risks. This study employed various bioassays to evaluate its biological effects and tracked its environmental presence along the Yellow Sea coastline. Toxicity evaluations encompassed general (e.g., Microtox, algal assays) and specific effects, such as phytotoxic responses in algal species, glucocorticoid receptor activity (via GR-CAFLUX), and estrogenic activity, alongside endpoints for reactive toxicity, including immobilization in copepods and fish embryo mortality. Tetracene was found to significantly activate the glucocorticoid receptor without eliciting estrogenic responses. Exposure induced algal growth suppression (EC50: 1.31–2.39 mg L⁻¹) and copepod mortality (EC50: 0.52 mg L⁻¹), with *Cyprinodon variegatus* exhibiting acute lethality (LC50: 1.2 mg L⁻¹). Monitoring results revealed concentrations surpassing 4,000 ng g⁻¹ (dry mass) in Nantong, with levels above 200 ng g⁻¹ (dry weight) in Qinhuangdao and Huludao. These findings emphasize tetracene's ecological risks and highlight the need for targeted environmental management and remediation efforts.

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2.07.P-Th069 Alkylation and Halogenation have Position-dependent Effects on PAH Potency for Aryl Hydrocarbon Receptor Activation and Early Life-stage Toxicity in Zebrafish (*Danio rerio*)

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental chemicals originating from natural and anthropogenic sources. Certain PAHs induce toxicity by dysregulating the aryl hydrocarbon receptor (AhR) which has been linked to dose-dependant mortality and developmental malformations such as pericardial and yolk sac edemas, and spinal curvatures. The US Environmental Protection Agency (EPA) has designated 16 unsubstituted parent PAHs as priority pollutants. These EPA 16 PAHs have become widely accepted as standard test compounds and have been the focus of most PAH toxicity studies to date. As a result, the effect of substitutions such as alkylation and halogenation on PAH toxicity are not well characterized despite substituted PAHs being ubiquitous in some environmental matrices. This research aims to (1) characterize the effects of halogenation or alkylation on potency for dysregulating the AhR in vitro, and (2) determine whether differences in potency for in vitro AhR activation result in differential embryotoxicity. Potency for activation of the zebrafish (*Danio rerio*) AhR2 was quantified using a standardized in vitro AhR transactivation assay. Toxicity was assessed by exposing freshly fertilized zebrafish to each parent, alkylated, or halogenated PAH via microinjection. First, benz[a]anthracene (BAA) and three alkyl homologues: 4-MBAA, 8-MBAA, and 7,12-DMBAA were tested. Alkylation had a position-dependent effect on potency for AhR activation. The most potent alkyl PAH was 8-MBAA with a 6.9-fold increase in potency, while 4-MBAA increased potency by 1.4-fold and 7,12-DMBAA decreased in potency. Similar relative potencies were observed in vivo. Halogenation also had position-dependent effects on potency for AhR activation. Anthracene, the parent compound did not activate the AhR of zebrafish, but 9/11 halogenated PAHs did with EC50s as high as 1100 nM, and as low as 7 nM which is as potent as the prototypical AhR agonist TCDD (6 nM). The rank order of potency for early life-stage mortality matched the rank order of potency for AhR activation. These results show that substitution of PAHs can increase the potency of PAHs, and in vitro transactivation assays can be used to accurately investigate the effects of substitution on potency. Due to the abundance of PAHs in the environment and the potential for increased toxicity inclusion of substituted PAHs in future research could be essential to accurately assess risks posed by PAHs.

2.07.P-Th070 ONE-BLUE Project: Integrated Assessment of Contaminants of Emerging Concern (CECs) and Their Impacts in Marine Ecosystems: Mediterranean Case Study

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The Mediterranean Sea is home to thousands of marine species, many of which are unique to the region and found nowhere else on Earth. Despite covering less than 1 percent of the world's ocean surface, the Mediterranean harbours 10 percent of all known marine biodiversity, making it an ecosystem of exceptional ecological significance. However, it is also among the most populated, as well as polluted and threatened marine areas in Europe, facing pressures from coastal urbanization, intense maritime traffic, plastic pollution, emerging contaminants, and climate change. Together, these factors are compromising the health and resilience of this fragile and vital ecosystem.

To address these concerns, the Mediterranean has been chosen as a key case study for the European ONE-BLUE project. This collaborative initiative seeks to close critical knowledge gaps on the presence, behaviour, and impacts of CECs in the marine environment through a multidisciplinary approach that integrates targeted and non-targeted chemical analyses, bioassays, and microbial assessments. The findings will contribute to a comprehensive understanding of how these contaminants move through and affect marine ecosystems.

The Mediterranean case study includes various study areas across the Spanish coast, the Tyrrhenian Sea, the northern and central Adriatic Sea, the Ionian Sea, and the Aegean Sea. Sampling areas cover diverse environments such as sites near fish and shellfish farms, industrial zones, shipyards, domestic and industrial wastewater treatment facilities, and river estuaries. Water and sediment samples are collected along the coastline and at offshore stations across various depths. Chemical analyses and toxicological tests will be conducted to assess the profile and concentration of CECs in both the water column and sediments.

Additionally, biota samples from different levels of the marine food web will be examined, including plankton (collected using 53, 125, and 200-micron nets), molluscs, fish, stranded sea turtles, and stranded marine mammals. Temporal trends in synthetic chemicals will also be studied in stranded cetacean samples from 2022 and stranded turtle samples from 2012, helping to assess the impact of mitigation measures and the phase-out of specific pollutants. Finally, to complete the analysis of the marine food chain, blood samples from Scopoli's shearwater nestlings will be collected just before their fledging period at six colonies across the Mediterranean Sea.

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2.07.P-Th071 Spatial and Temporal Distribution of Metals in the Salt River Catchment, Cape Town Zikhona Menze, SETAC member, South Africa

The Liesbeek and Black Rivers are two major urban tributaries of the Salt River. The latter enters the ocean at Paarden Island, Table Bay. A multitude of pollution sources are found along the banks of these rivers, including industries, houses, sewage treatment plants, golf courses and informal settlements. The catchment has been severely modified and canalized, increasing flooding events and sediment loads downstream. This catchment is seen as potentially in the worst ecological condition of all Cape Town catchments and its condition is seemingly deteriorating consistently. However, the current degree of pollution is unknown for this catchment. This study investigates the spatial distribution of metals as well as temporal distribution, through comparison of new data with previous data from 2006, and comparison of wet and dry seasons. Sediment samples were acid digested and a range of metals were analysed with an ICP-MS. Preliminary results show that sections of both the Liesbeek and Black Rivers are heavily polluted with metals (e.g., Al and Fe) and that metal levels have significantly increased since 2006 due to increases in land use. Metal concentrations were also generally higher during the dry season, most likely due to the slower current. The Salt River itself has proven difficult to study as it is entirely canalized, which indicates that potentially high loads of polluted sediment are carried straight to the lagoon. This study will form an

important basis for further studies on the impacts of aquatic pollution on freshwater, coastal and marine biota.

2.07.P-Th072 Ecological Risk Assessment Post Tourist Season at the Croatian UNESCO Heritage Island of Hvar: Student STEM Research Immersion Program Promotes Environmental Stewardship and Community Outreach

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To assess the potential adverse anthropogenic effects on sea water quality at the end of the tourist season, a comprehensive ecological risk assessment was initiated at the Island of Hvar, Croatia in September 2024. Composite Integrative Passive Samplers (CIPS) were deployed for ten days at 16 sites that included two main town center bays, several public beaches, two ferry ports, boat-maintenance bay, a popular dive center, and secluded beaches along the 65-km island, which is one of the most popular summer tourist destinations in the world and a UNESCO World Heritage site. CIPS captures both water- and lipid-soluble contaminants, allowing measurement of time-weighted concentrations of industrial and persistent organic pollutants; the quantitative targeted chemical analysis using Liquid chromatography mass spectrometry is used to characterize over 400 known chemicals, including many carcinogens, mutagens, and persistent organic industrial pollutants: polychlorinated biphenyls, polyaromatic hydrocarbons, plasticizers, pesticides, herbicides, and plasticizers. Besides sea water chemical profiling and standard sea water measurements (pH, salinity, temperature, dissolved oxygen, conductivity / salinity), we collected native species of Mediterranean mussels (*Mutilus galloprovincialis*) and sea urchin (*Arbacia lixula*) within 25 meters of each sampling site. Animal tissues will be used to characterize body burdens of targeted chemicals and correlate the data with the site-specific CIPS chemistry profiles; moreover, animal tissues will be utilized for metagenomic analysis, quantifying the presence of bacteria, viruses, parasites, and other microbes. Pending our chemistry and metagenomics results, several animal tissues collected at specific sites will be used for selected gene expression profiling to identify biomarkers of exposure to complex industrial chemical mixtures. Our goal is to characterize and quantify the human activity impact on ecology and sea water quality at this popular and unique island.

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2.07.P-Th073 Effects of Surfactants on *Pelagia noctiluca* Ephyrae, a Promising Model Organism in Marine Ecotoxicology

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The effects of marine pollutants are usually assessed with standard ecotoxicity tests relying on a limited number of marine model invertebrates. Expanding the range of sensitive and ecologically relevant species is important to represent the full impacts of pollutants in marine ecosystems. Cnidarians are widely distributed in oceans and integral components of marine food webs and, among them, jellyfish are exposed to pollutants via multiple pathways, representing promising models for ecotoxicity studies. So far, few jellyfish species have been investigated for this purpose, focusing on the ephyra stage as suitable due to its susceptibility to toxic compounds.

Here we explore the use of the mauve stinger *Pelagia noctiluca* (ephyra stage) as a new model organism in ecotoxicology, by evaluating its responses to two common surfactants: Sodium Dodecyl Sulfate (SDS) and Tween 20.

After setting the optimal test conditions through preliminary tests, *P. noctiluca* ephyrae (10 ind/well) were exposed for 48h to reference toxicants (SDS: 0.1 -50 mg/L, Tween 20: 0.0001 -10%). At 24h and 48h, both lethal (mortality) and sub-lethal endpoints (behavior) were evaluated. Swimming behavior was determined both quantitatively, as the number of pulsations made by each ephyra within one minute, and qualitatively by tracking the ephyrae swimming path within the well and classifying the type of lappet movements (complete and wide contraction, altered contraction or immobility).

After 24h of exposure to SDS, 100% of mortality was observed at the concentration of 50 mg/L, whereas after 48h the same effect occurred at 10 mg/L. For Tween 20, all the ephyrae were dead at 0.1% after both

24h and 48h of exposition. LC50 values for SDS were 7.5 ± 0.6 mg/L and 6.1 ± 0.8 mg/L after 24h and 48h, respectively, while for Tween 20 LC50 values corresponded to $0.045 \pm 0.00\%$ at 24h and 48h. A significant reduction in the pulsation rates was found in the ephyrae exposed to low concentrations of both surfactants. Alterations in the swimming quality were further recorded with altered lappet contractions or ephyrae pulsations without movement.

Our findings show acute toxicity of SDS and Tween 20 to *P. noctiluca*. The high sensitivity of the ephyrae of this species compared with other established model organisms (e.g., *Artemia franciscana*, *Daphnia magna* and *Amphibalanus amphitrite*) underscore their potential use in ecotoxicological research, improving the impact assessment of pollutants on marine life.

2.07.P-Th074 Ecotoxicological Effects of Metal Mixtures from Offshore Wind Turbine Galvanic Anodes on Blue Mussels *Mytilus edulis*

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Offshore wind energy is a pivotal strategy for achieving carbon neutrality in electricity generation. Offshore locations offer the advantages of higher wind speeds and reduced spatial competition with human activities compared to onshore sites. However, the expansion of offshore wind farms to meet the 2050 carbon-neutral targets introduces potential pressures on marine ecosystems, particularly through trace metal emissions from galvanic anodes cathodic protection anti-corrosion systems. While zinc and aluminum are the primary metal ions emitted, other trace metals such as cadmium, lead, indium and gallium have also been reported as key components in these alloys. Despite well-documented toxic effects of zinc, aluminum, cadmium, and lead on marine organisms, such as oxidative stress, reduced growth potential, and DNA damage, little is known about the toxicity of gallium and indium, either alone or in combination with other metals. In a laboratory experiment, we are investigating ecotoxicological effects of a mixture of trace metal elements (Al, Zn, Cd, Pb, Ga, and In), emitted from galvanic anodes on the blue mussels *M. edulis*. Thereby the mussels will be exposed to increasing dissolved metal concentrations, achieved by dissolving pure chloride metal salts in relative proportions to the composition of aluminum based galvanic anodes. The lowest exposure concentrations will be comparable to those currently obtained around wind farms in the North Sea. Metal bioaccumulation, energy stores, oxidative stress enzyme activity, metallothionein induction, and expression of defense-related genes in gill and digestive tissues will be assessed after 1, 3, 7, and 14 days. Preliminary results after 7 days exposure show indication of some physiological effects of the exposed metals. This study will contribute to understanding the biological impacts of cumulative metal emissions from offshore wind farm anodes, enhancing environmental models and guiding sustainable planning to mitigate ecological effects while balancing offshore wind energy's socio-economic and environmental benefits.

2.07.P-Th075 Effects of Underwater Noise on Behavior and Physiological Stress Responses in Baltic Sea Blue Mussels (*Mytilus spp.*)

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Underwater noise pollution is a growing concern in marine ecosystems, with potential effects on the physiology and behavior of marine organisms. In the Baltic Sea, blue mussels (*Mytilus spp.*) are essential habitat-forming species that may be vulnerable to such disturbances. This study aimed to investigate the effects of vessel noise on blue mussel behavior and physiological stress responses, focusing on simulated traffic scenarios.

Baltic Sea blue mussels were exposed to two different noise treatments in controlled aquaria for a three-day period: (1) moderate boat traffic, and (2) intense boat traffic.

These treatments involved playback of field-recorded boat passage noise, presented at randomized intervals interspersed with periods of ambient noise. Traffic rates were derived from real-world data, combining AIS-based vessel tracking and hydrophone recordings from the Archipelago Sea. Behavioral responses were evaluated by continuously monitoring valve gape activity. Biochemical stress responses were evaluated by analyzing antioxidant enzyme activities as indicators of oxidative stress, lipid peroxidation as a marker of cellular oxidative damage, and acetylcholinesterase (AChE) activity as an indicator of neurotoxic effects in both exposed and control mussels.

Mussels exposed to noise treatments exhibited significant changes in valve gape behavior. Prolonged valve gape closure was observed during exposure periods with frequent boat passages, while periods

containing more randomly occurring boat passages induced more variable gape patterns, suggesting differential responses to noise type and intensity. Observed biochemical responses suggest that noise exposures trigger significant cellular stress. Additionally, change in AChE activity may indicate a neurophysiological response possibly linked to both oxidative stress and valve gape behavior. The observed behavioral and biochemical responses suggest that underwater noise, even at varying intensities and patterns, induces stress in blue mussels, likely affecting their health and ecological role. These findings underscore the importance of recognizing noise pollution as a significant stressor in the Baltic Sea marine environment. They enhance understanding of how noise can contribute to cumulative impacts that may threaten the stability of the ecosystem.

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2.07.P-Th076 Comparative Environmental Health Status Assessment Through Cell and Tissue Level Biomarkers in Wild Mussel and Oyster Populations from Locations with Different Anthropogenic Impact in the Basque Coast

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In the last decades, the distribution of *Mytilus* spp. in the Atlantic Ocean has considerably changed, and different mortality events have been reported in the area leading to a decrease in mussel population possibly due to multifactorial reasons (i.e., parasites prevalence, global warming, alien species pressure, anthropogenic impact among others). This raises the need for a change in the employed sentinel organisms, and oysters (*Magallana gigas*) have been considered a suitable alternative to mussels. Within this context, the aim of the present study is to determine if general stress cell and tissue level biomarkers are comparable in *Magallana gigas* and *Mytilus galloprovincialis* for environmental health assessment. For this purpose, histological, histochemical and chemical measurements were carried out in mussels and oysters gathered in three different sampling points of the coast of the Basque Country. Two of these sites are located at the mouth of the Bilbao estuary (Arriluze and Arrigunaga), which has been historically subjected to industrial wastes and presents a poor environmental quality, while the other site, Plentzia, is located in Butroe estuary, considered for this study as a reference site with low to moderate levels of pollutants. Preliminary results show that some of the biomarkers previously measured in mussels can be similarly applied in oysters, like atrophy of the digestive epithelium, connective to digestive tissue ratio or lipofuscin volume density, whereas others like adipogranular cell density, neutral lipid volume density and lysosomal biomarkers cannot be directly compared and need to be studied and adapted or modified. Additionally, mussels show higher number and more relevant histopathological alterations, such as greater prevalences of granulocytomas and haemocytic infiltrations. Overall, a worse health status is noted at the estuary of Bilbao compared to Plentzia, with higher levels of atrophy and lipofuscins; which is consistent with a higher presence of pollutants. Currently, knowledge on the responsiveness of oyster biomarkers is more limited than in mussels and further research is needed to establish baseline values to be considered as suitable candidates for their substitution as sentinel organisms in the Basque Coast.

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2.07.P-Th077 Acute Toxicity of Lithium to Marine Organisms of Different Biological Complexity

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Lithium (Li) is an alkali metal of emerging concern, mainly due to its increasing use to produce Li-ion batteries. However, little knowledge is available regarding its effects on marine life. In this work, the acute toxicity of Li was determined in different key marine species (bacteria *Vibrio fischeri*, copepod *Acartia tonsa*, shrimp *Palaemon Elegans* and sea urchin *Paracentrotus lividus*). Experimental concentrations were selected according to Fibonacci scale: 0, 1, 5, 13, 34, 89, 233, 610 and 1597 mg/L. Li toxicity by Microtox® assay was based on the reduction of the bioluminescence in *V. fischeri* after 15 minutes of exposure. Copepods and shrimps were used for the exposure experiments in three replicates of 5-8 individuals each and mortality was checked daily for 2 days (copepods) and 4 days (shrimps) for the calculation of the lethal concentration (LC) values. Sea urchin embryos were exposed for 48 h and the size increase of larvae and their effect concentration (EC) values were calculated. Malformations were also

recorded and integrated in a Toxicity Index (TI), depending on the severity. Finally, the capacity to undergo the developmental program was studied by the application of different indexes. Based on the results obtained, the EC50 value obtained by the Microtox® assay was 3012.98 (CI: 2741.76 3311.035) mg/L, values much higher than environmentally relevant concentrations in oceans (several hundred µg/L). Copepods showed the highest sensitivity with an LC50 of 22.433 (CI: 11.508 38.942) mg/L after 48h. The EC50 values obtained for the sea urchin embryos revealed that the TI (43.629 (CI: 33.430 57.579) mg/L) presented a higher sensitivity than size increase (89.959 (CI: 58.437 136.266) mg/L). Finally, the development test results showed that high Li concentrations (233 and 610 mg/L) stopped development at morula, or even at cleavage stage (1597 mg/L). Upon lower levels (5, 13, 34 and 89 mg/L), more embryos reached pluteus stage, although malformations were detected, thus, sub-lethal levels should not be underestimated. These findings give insight into the toxicity of Li to different marine species and contribute to assess possible effects at different taxon to understand the sensitivity of species to this emerging pollutant. Moreover, the disparity in results between species shows the need to further investigate the effect of Li, as well as potential changes in sub-lethal/ chronic exposure scenarios.

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2.07.P-Th078 Impact of Polycyclic Aromatic Compounds and Oil Exposure on the Righting Behavior of Two Gastropod Species

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Of the 1000s of components within crude oil, polycyclic aromatic compounds (PACs) are thought to be the primary driver of toxicity. Due to the complexity of a crude oil spill, stakeholders must rely on mathematical models to predict and assess the impacts of a spill on aquatic life. Oil spill models rely on species sensitivity distributions (SSDs) to determine species at risk if exposed, and there is very limited data available on the sensitivity of gastropods to petroleum contaminants. Distributed throughout the North Atlantic, periwinkle snails are found in high numbers in intertidal zones, and field observations made during the Sea Empress and Amoco Cadiz oil spills suggest that they may be sensitive to hydrocarbon exposure. Abalone are a slow growing herbivorous gastropod that historically served as a subsistence food for indigenous peoples along the west coast of North America. Due to overfishing, and pressure from disease and predator population booms, populations of abalone have declined significantly, and there is limited data available to determine if PAC pollution could be an additional threat. The aim of the study was to generate toxicity data to determine the sensitivity of two gastropod species, the common periwinkle snail (*Littorina Littorea*) and the red abalone (*Haliotis rufescens*) to PACs using a sublethal behavioral endpoint, and to see if the effects observed in single compound exposures are predictive of whole oil effects. The PAC test solutions were generated using passive dosing with polydimethylsiloxane (PDMS) O-rings left to equilibrate in 1L glass jars for 24 hrs. For the oil exposures, water accommodated fractions (WAFs) of crude oil were prepared using a 1 g/L loading in natural seawater with 20 hrs mixing (20% vortex) and 4hrs settling. Adult snails and the juvenile abalone were then exposed for 24 hours in aerated 1L glass jars and were subject to a righting assay. For the righting assay, the gastropods were placed on their back in a crystalizing dish with clean seawater and left undisturbed for 1hr. Results suggests that righting behavior assay was a repeatable and reliable way to test PAC toxicity in gastropods. It was also very sensitive endpoint, and abalone were more sensitive to PAC exposure than the periwinkle snails. The data generated in this study can be used to better understand the sensitivity of gastropods to PAC exposure, and to assess the impact of oil spills on underrepresented test species.

2.07.P-Th079 Marine Environment Impacts from Space Launch Activity

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In recent years, there has been a significant rise in global space launches, with rockets primarily delivering commercial satellite payloads into orbit. While the sector is currently dominated by the US and China, spaceports are emerging within the OSPAR region (North East Atlantic), with active or planned developments in the UK, Norway, Sweden, and other countries. By 2030, several spaceports could be operational, but the exact number remains to be determined. During launch, space launch vehicles jettison various components, such as stage 1 and 2 engines and nose cones (fairings), which can be deposited into the marine environment. Additionally, upon re-entry, further debris from rockets and payloads can be deposited into the ocean. Typically, these jettisoned parts are not recovered, and have the potential to

cause a range of environmental impacts. Current definitions of long-term space sustainability focus largely on the orbital environment, neglecting the Earth-based impacts, particularly those related to the marine environment. Evidence of the environmental effects of space launch activity, particularly concerning marine debris, remains limited and there are significant knowledge gaps. A literature review and interviews with experts in the industry was used to gather insights on the potential impacts from jettisoned rocket parts. Some of the identified impacts include: creation of marine litter from debris, release of contaminants such as fuel and other chemicals (some of which are Substances of Very High Concern under EU REACH), and smothering of organisms on the seabed. There is no global or regional reporting requirement for deposition of rocket parts into the ocean and international instruments such as OSPAR, London Convention/London Protocol and UNCLOS do not specifically address this issue. Options to improve information gathering, reporting, best practices to prevent and reduce marine environmental effects and international cooperation and information exchange should be considered.

2.07.P-Th080 Biomakers Assesment for Matrices Artificially Contaminated with Dichlofluanid

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Antifouling paints are used to combat biofouling on submerged surfaces. These paints contain biocidal compounds that can be extremely harmful to aquatic ecosystems. Among the different biocides present in antifouling paints, dichlofluanid stands out, also known as N-dichlorofluoromethylthio-N', N'-dimethyl-N-phenylsulfamide (C₉H₁₁Cl₂FN₂O₂S₂). Little is known about how this active ingredient affect marine organisms. Therefore, it is important that studies on the topic are conducted to evaluate the biological effects of this contaminant. The aim of this study was to evaluate the effect of dichlofluanid at the cellular and biochemical level, using bivalves exposed to seawater and sediment contaminated with this biocide, while also considering sediments with two different concentrations of organic matter. Exposure tests were carried out in water with bivalves species *Perna perna*, and in sediment with the bivalve *Anomalocardia flexuosa*. The neutral red retention time assay was used in *P. perna*, while biochemical biomarkers such as DNA damage, LPO, GSH, GST, EROD, AChE and GPx were analyzed in both species. Particle size, carbonate content and organic matter analyzes were also conducted on the sediments. In water, an effect was observed at higher concentrations in the neutral red assay, indicating a loss of lysosomal stability. For biomarkers, changes were observed in the digestive glands and gills. In sediment, effects were noted in both organs, with more significant changes in sediments with higher levels of organic matter. Based on the results of these studies, environmental concentrations of dichlofluanid are likely to cause effects to the organisms studied.

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2.08.P New Developments in Sediment Ecotoxicology and Risk Assessment

2.08.P-Mo157 Effect-Based Trigger Values for a Mixture of Aryl Hydrocarbon Receptor Agonists in Sediments Derived from In Vitro Bioassays

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Target chemical analysis is commonly used to monitor persistent toxic substances in the marine environment; however, it cannot assess contamination by unknown compounds. To address this limitation, effect-based monitoring (EBM) utilizes in vitro bioassays to evaluate the biological activity of environmental samples, including the mixture effects of known and unknown compounds. Implementing EBM requires the establishment of effect-based trigger values (EBTs) to differentiate between acceptable and unacceptable levels of persistent toxic substances. While EBTs studies have primarily focused on the water environment, research on sediments remains limited. This study aimed to derive appropriate EBTs for aryl hydrocarbon receptor (AhR) activity in sediments using H4IIE-luc bioassays and to evaluate AhR activity in highly industrialized coastal sediments based on the derived EBTs. The first EBTs method (Criteria 1) was to obtain instrumental analysis data, such as BaP equivalent concentrations, using the predicted no-effect concentration (PNEC_{sediment}) values and the relative potency values of 20 individual AhR agonists. The EBTs was calculated by dividing the sum of BEQs by the total number of compounds. PNEC_{sediment} values were determined using the KOC values of each AhR agonist and the minimum

concentration inducing AhR-mediated potency. The second EBTs method (Criteria 2) was a value incorporating cytotoxicity, which was calculated using the predicted baseline toxicity (IC10) and EC10 values for 20 AhR agonists. The EBTs derived using Criteria 1 was 707 ng BaP-EQ g⁻¹ dw, and the EBTs derived using Criteria 2 was 200 ng BaP-EQ g⁻¹ dw. The AhR activity in sediments from South Korea were evaluated using these EBTs. The BaP-EQ_{bio} concentrations in sediments from Ulsan Bay, Lake Sihwa, Masan Bay, Yeongil Bay, and Busan Bay ranged from 610 12,000, 580 1,800, 33,000 45,000, 1,700 220,000, and 1,300 5,400 ng BaP-EQ g⁻¹ dw, respectively. When evaluated using Criteria 1 EBTs, Ulsan Bay, Masan Bay, and Yeongil Bay sediments exceeded the threshold. On the other hand, when evaluated using the Criteria 2 EBTs, 14 coastal sediments exceeded the EBTs. These results suggest the need for further evaluation of AhR activity and continued monitoring of AhR agonists in these regions. Future efforts should include a broader range of AhR agonists in EBTs calculations, preceded by effect-directed analysis studies to identify and characterize additional AhR agonists in sediments.

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2.08.P-Mo158 Toxicity Assessment of Freshwater Sediments: Proposing the European Amphipod *Gammarus fossarum* for the Development of New Standardised Bioassays

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Freshwater sediments act as sinks for various contaminants, including metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and emerging pollutants like pharmaceuticals. These contaminants accumulate over time, posing risks to benthic organisms and creating challenges for sediment management. Addressing this issue is critical for understanding ecosystem health and improving sediment management practices.

This study aims to standardise bioassay protocols for assessing sediment toxicity using the European amphipod *Gammarus fossarum*. The goal is to evaluate the biological effects of contaminated sediments on feeding behavior and reproduction while considering confounding factors of sediments such as granulometry and organic carbon content.

To tackle these challenges, 140 sediment samples were tested for feeding rate, and 84 for reproduction, using protocols adapted from standardised methods. Physico-chemical analyses of sediments, including particle size distribution and contaminant concentrations, were conducted in parallel. Statistical analyses linked sediment characteristics to biological endpoints to derive threshold values and refine risk assessment.

Preliminary results show minimal influence of granulometry and organic carbon content on the endpoints, enabling threshold value proposals (2 g/gammarid/day for feeding, 10 embryos/female for reproduction). However, artificial sediment used as control showed reduced feeding and reproduction rates compared to uncontaminated natural sediments, raising concerns about their suitability. Feeding rate proved to be a more sensitive indicator of sediment toxicity than reproduction, although both endpoints complement each other in assessing impacts.

This research highlights *G. fossarum* as a promising bioindicator for sediment toxicity. The findings allow for the development of standardised bioassays and thresholds, contributing to sediment quality assessments. These insights can inform regulatory frameworks and sediment management strategies, advancing our understanding of freshwater ecosystem health and aiding in sustainable sediment management practices. Therefore, this work is aligned with the session 2.10 « New Developments in Sediment Ecotoxicology and Risk Assessment ».

2.08.P-Mo159 “In Vivo” ECOD Assay: A Proxy to Unveil Biotransformation in Sediment-Dwelling Invertebrates.

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The ECOD (7-ethoxycoumarin-O-dealkylation) "in vivo" assay is a valuable tool for assessing the biotransformation capabilities of sediment-dwelling invertebrates, specifically focusing on Cytochrome P450 (CYP450) activity. This study presents an optimized method for performing the ECOD assay with sediment-associated hydrophobic compounds, using the polychaete *Capitella teleta* as a model organism.

The ECOD assay, traditionally used in "in vitro" setups, has been adapted for "in vivo" conditions to provide a more accurate quantification of CYP450 activity over time. This adaptation is particularly relevant for sediment-dwelling organisms, which are often exposed to contaminants through sediment ingestion rather than water.

C. teleta individuals were exposed to sediments spiked with a mixture of PCBs (#52, #153, #209) or Sertraline at concentrations of 50 and 100 µg/g, respectively. The exposure lasted for 14 days, after which the worms were transferred to a multi-well plate containing 7-ethoxycoumarin solution and spiked sediment for continuous exposure. Fluorescence detection was used to measure CYP450 activity, with samples collected every 15/30 minutes.

The optimized assay successfully measured CYP450 activity during sediment exposure, showing expected trends for the different contaminants. PCBs, known inducers of CYP450 activity, resulted in increased fluorescence detection compared to control values, while Sertraline, an inhibitor of CYP450, led to a decrease in activity. The results indicate significant differences in CYP450 activity among treatments, aligning with the known effects of the contaminants.

This study demonstrates that the "in vivo" ECOD assay can be effectively performed with sediment-associated hydrophobic compounds and sediment-dwelling organisms. The optimized method provides a powerful tool for time-dependent quantification of CYP450 activity and biotransformation rates in sediment. Further investigations with different species and contaminants are warranted to assess the broader applicability of the optimization.

2.08.P-Mo160 Overcoming Challenges in Chronic Toxicity Testing of Sediment-Dwelling Organisms: Validating Spiked Sediment Studies for a Variety of Species across Diverse Taxonomic Groups

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Under the EFSA Aquatic Guidance (2013), which has been noted and used since 2015 for the aquatic risk assessment of plant protection products (active substances, new and renewals; formulated products under zonal authorizations or country registrations) in the EU, there are various Tiers and options offered for testing and refinements for aquatic organisms exposed via the water phase.

However, for sediment dwelling organisms exposed to contaminated sediment, higher tier options for refinement are significantly limited in comparison. In practical terms, the Tier 3 mesocosm option is by default not realistic as such studies performed with spiked sediment to achieve a worst-case/realistic exposure regime are very challenging. Similarly, pulse exposure (Tier 2C) is also not a realistic refinement option due to the very nature of the exposure expected (accumulation in sediment). Therefore, the only practical options are based on additional single species laboratory testing followed by data analysis i.e., geometric mean approach (Tier 2A) or species sensitivity distribution (SSD) approach (Tier 2B). These approaches require a dataset of toxicity endpoints from different species preferably belonging to different taxonomic groups.

We are here discussing a case study demonstrating that even the above option is realistically very challenging: identifying a variety of sediment dwelling organism species and performing successful testing under GLP conditions that result in valid studies can be a complicated task. There are many factors contributing to this. Mainly, the lack of standardized methodologies, particularly for chronic/reproductive toxicity testing for various species and the need of adapting existing guidelines and relevant validity criteria to different species. In this case study the attempt to test 17 different sediment dwelling species (from insect, crustacean, polychaete, nematode and bivalve taxonomic groups) at three different contract research organizations is presented. All the practical problems encountered, such as acquisition of test organisms, success rate and the failures of validity criteria or the lack of possible valid adaptation and interpretation of existing guidelines are presented. With this presentation we hope that the necessity of further standardized methodology and guideline development is highlighted for sediment dwelling organisms (with spiked sediment) toxicity testing.

2.08.P-Mo162 Development of a Conceptual Model for Sediment Dynamics and Pollutant Distribution in a Connected Pond System

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Reinfelder Teiche comprises a system of connected ponds which are located in the centre of the city of Reinfeld, Northern Germany. Surface run-off from urban areas and agricultural fields as well as emissions from domestic sewage treatment plants are likely to have an impact on the chemical quality of water and sediment and the biodiversity of the area. Various measures to improve the biodiversity of the pond system have been much debated but postponed up to now, because the impact of potential chemical,

biological and human stressors on the pond s ecosystem is not well known.

In order to better understand the dynamic of the accumulating sediment and its contamination, sediment cores have been taken at all ponds, profiles of heavy metal contamination and ecotoxicological effects have been measured with depth. Chemical contamination was measured at the different water systems and sedimentation rates were determined. In each of the 5 most important ponds, the upper 3 cm of sediment surface layer of the 7 most important ponds was analysed by a set of 4 bioassays and a nematode-based in-situ index (NemaSPEAR[%]-index). A more detailed chemical analysis of sediment pollutants and dissolved pesticide concentrations and effects in inflowing waters, a conceptual model for the ponds will be set up to help improving system understanding and providing a first basis for targeted measures.

2.08.P-Mo163 Distribution of Potentially Toxic Elements in Sediments in Millstream Creek, Langford, BC, Canada

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Westshore Motorsports Park (WMP) founded in 1954 near Victoria operated as a racing track until its closure in 2022. The area is currently being developed as a housing complex and business park. There is concern about its legacy in the form of potentially toxic elements (PTEs) such as arsenic, cadmium, chromium, copper, mercury, nickel, lead and zinc emissions from the motor cars that used to race on the tracks. Twenty-nine sediment samples were collected from Millstream Creek, which flows past the WMP, to assess how the surrounding land use influence metal distribution in the sediments. Samples were collected along the stream at locations upstream (Area A), immediately downgradient (Area B), and further downstream (Area C) of the WMP. Total metal concentrations were determined by x-ray fluorescence and the physicochemical properties of surface water were also evaluated. There was a statistical difference in pH with Area B, immediately downgradient of WMP having the highest mean concentration (pH=7.63). The concentrations of the arsenic, cadmium, and mercury in all the sediment samples were below detection. Chromium, copper, and zinc in some of the sediments samples exceeded the Canadian Council of Ministers of the Environment (CCME) interim freshwater sediment quality guidelines (ISQG). The elevated chromium concentrations could be attributed to the relatively high chromium background concentrations on Vancouver Island. Copper, nickel, and zinc were comparatively higher in Area B with the mean concentrations of copper (49.6 mg/kg) and zinc (125 mg/kg) exceeding the CCME ISQG. The statistically significant higher concentrations in Area B could be attributed to impacts from the WMP, albeit the presence of a highly vegetated riparian zones may limit the migration of contaminants into the creek. The results of the human health and ecological risk assessment including the influence of a riparian zone along with an exploration of phytoremediation as a nature-based solution for easing the transition toward regenerative development will be presented.

2.08.P-Mo164 Influence of Sediments Spiked with “Smart” Anti-corrosion Nanoadditives in *Hediste diversicolor*: Biomarkers and Bioaccumulation

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The corrosion of immersed metallic structures generates a significant economic problem, which can be reduced using corrosion inhibitors (CI) that retard this process. However, most CIs are toxic to the biota, requiring eco-friendly alternatives. ZnAl layered double hydroxides (LDH) have been used to immobilize different CIs to develop smart or stimuli-responsive anti-corrosion nanoadditives that can reduce toxicity and increase corrosion protection over time. This study aimed to assess the toxicity of four CIs (2-mercaptobenzothiazole (MBT), benzotriazole (BTA), sodium gluconate (SG) and sodium nitrite (NaNO₂)), the respective nanoform (LDH-MBT; LDH-BTA; LDH-SG; LDH-NO₂), and the raw nanomaterial (LDH-NO₃) through biochemical biomarkers and bioaccumulation in the polychaete *Hediste diversicolor*. The dissolution of nanomaterials was also quantified in artificial saltwater (ASW) and sediment. Juveniles of *H. diversicolor* were exposed individually in 200 mL flasks containing 6 cm of contaminated sediment in 3 different concentrations (0.2; 2; 20 mg kg⁻¹) and 50 mL of ASW for 10 days. After the exposure, the organisms were preserved to biochemical biomarkers and bioaccumulation determination, while the sediment and water were appropriately stored for Zn²⁺ and Al³⁺ quantification. Acetylcholinesterase (AChE), lipid peroxidation (LPO), catalase (CAT), and glutathione-S-transferases (GST) were tested for all the nanostructured and soluble forms of the four CIs. The findings of the biomarkers indicated that MBT, LDH-MBT, LDH-BTA, and LDH-NO₃ caused mainly neurotoxicity. Bioaccumulation of Al³⁺ and Zn²⁺ was observed for all nanoforms, apart from LDH-SG (not measured

for technical reasons). In the overlying ASW, dissolved Zn was increased only in 20 mg kg⁻¹ of LDH-MBT and LDH-BTA. Regarding dissolution in sediment, there was a decrease of Al in all concentrations of raw LDH-NO₃, as well as of LDH-MBT, and LDH-NO₂, in 0.2 and 2 mg kg⁻¹ of LDH-SG and in 2 mg kg⁻¹ of LDH-BTA. Reduced Zn concentrations were also observed in all concentrations of LDH-NO₂ and LDH-NO₃, while increased concentrations occurred in 20 mg kg⁻¹ of LDH-SG and LDH-BTA, and no difference in LDH-MBT. These findings on the effects and bioaccumulation of such innovative smart nanomaterials and their dissolution in sediment demonstrate their potential as eco-friendly alternatives. Future studies must confirm these results in scenarios where the nanoadditives are incorporated into coatings.

2.08.P-Mo165 Hediste Diversicolor Model for the Evaluation of Estuarine Sediment Toxicity Impacted by Sewage Treatment Plants

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River estuaries are vital transitional ecosystems that experience significant anthropogenic pressure, leading to the accumulation of pollutants in sediments. While the ecological impacts of treated sewage discharges on river sediments have been previously studied, estuarine ecosystems pose additional challenges due to their dynamic physico-chemical properties. To address threats to ecosystem resilience posed by sewage treatment plant (STP) discharges, novel ecotoxicological models that provide realistic, multi-level responses are essential.

This study employed the endobenthic ragworm, *Hediste diversicolor*, a widely distributed model organism, to evaluate the impacts of different size STPs on river estuaries. Ragworms were exposed for seven days to air-dried estuarine surface sediments collected during two distinct seasons from upstream, discharge, and downstream sites. We assessed toxicity endpoints at multiple levels of ragworm biological organisation, including primary behavioural responses at the beginning of incubation and acute toxicity, cellular changes, and biochemical alterations after the end of incubation.

The results revealed heightened stress behaviours in ragworms exposed to sediments from all discharge sites. Coelomic amoebocyte viability was significantly reduced at discharge and downstream sites compared to upstream sites in one of the large estuaries under study, highlighting the impact of sediment-associated contaminants on the health of benthic organisms. Preliminary data from acute toxicity tests using sediment elutriates with rotifers and analyses of non-polar pharmaceutical residues further support these findings. Moreover, sediment sand content emerged as a key stressor, strongly correlating with weight loss and reduced amoebocyte viability in ragworms. Oxidative stress biomarkers cyclooxygenase, superoxide dismutase, glutathione S-transferase, and acetylcholinesterase exhibited changes among sites. The findings emphasise the utility of a multi-level approach in applying *H. diversicolor* as a model organism for environmental monitoring. This study demonstrates the potential of integrating sediment characterization and multi-biomarker responses to improve ecotoxicological assessments in estuarine environments.

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2.08.P-Mo166 Distribution Characteristics and Potential Toxicity of Dioxin-Like Chemicals in Sediments from Busan Bay, South Korea

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This study investigates the contamination status of dioxin-like chemicals (DLCs) and their potential dioxin-like activities in sediments collected from Busan Bay, Korea. A total of 41 sediment samples were collected from North Harbor (n = 20), South Harbor (n = 12), and Gamcheon Harbor (n = 9). Of these, 5 sediment samples (N1, N2, N6, N7, and N10) exhibited significant AhR-mediated potency. The targeted 96 DLCs, including 17 polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), 22 polybrominated diphenyl ethers (PBDEs), 22 polychlorinated biphenyls (PCBs), and 35 polycyclic

aromatic hydrocarbons (PAHs) were detected at all sites. The concentrations of PCDD/Fs (17 210 pg g⁻¹ dw) were the highest in Gamcheon Harbor, while North Harbor showed elevated levels of PBDEs (1.6 280 ng g⁻¹ dw), PAHs (100 880 ng g⁻¹ dw), and PCBs (0.42 360 ng g⁻¹ dw) due to the influence of industrial complexes and port activities. To identify contamination sources, a positive matrix factorization (PMF) model and principal component analysis (PCA) were applied. The PMF model revealed that PAHs in Gamcheon and South Harbor originated mainly from petroleum, while those in North Harbor were attributed to diesel emissions. PCA results indicated that PCBs in the sediments were compositionally similar to industrial products such as Aroclor, Clophen A, and Kanechlor, and PCDD/Fs showed profiles similar to TPN 1993 and fly ash. Among the targeted DLCs, 35 specific compounds (17 PCDD/Fs, 7 PBDEs, 2 PCBs, and 9 PAHs) were detected in concentrations ranging from 43 to 160 ng g⁻¹ dw. A potency balance analysis was performed by comparing bioassay-derived TCDD-equivalent concentrations (EQs) with instrument-derived toxic equivalents to determine the contribution of specific DLCs to total TCDD-mediated potency. The specific DLCs contributed 30% and 42% to bioassay-derived TCDD-EQs (TCDD-EQ_{bio}) in N2 and N7, respectively, and explained more than 100% of TCDD-EQ_{bio} in N1, N6, and N10. PAHs in sediments were the primary contributors to dioxin-like activities. Among the PAHs, benzo[b]anthracene was identified as a major compound exhibiting TCDD-like potency in sediments from Busan Bay. These findings provide useful baseline data on the distribution of DLCs, their potential dioxin-like activities, and the relative contributions of individual DLCs in sediments from Busan Bay.

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2.08.P-Mo167 Exploring Coconut Husk as an Organic Matter Enrichment Alternative to Peat in Sediment Toxicity Test

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The utilization of standardized artificial sediment in toxicity assessments offers increased repeatability and enhances consistency across bioassays and laboratories. Artificial sediment is required for most OECD (Organisation for Economic Co-operation and Development) guidelines, particularly in studies assessing the impact of chemicals on benthic communities.

While peat is conventionally incorporated into artificial sediment formulations as the source of organic matter (OM), it is not a sustainable resource due to the long time it takes to form and the environmental impact of its extraction. Notably, peatlands, covering over 12% of Canada's land area, are pivotal in carbon sequestration, estimated to be 150-160 Gt of carbon. The extraction of peat leads to a carbon loss equivalent to 2.1 megatonnes of carbon dioxide emissions annually, accompanied by the potential release of other elements into the environment and aquifers. Consequently, the pursuit of less impactful alternatives is desirable, and aligns with the 2030 Agenda for Sustainable Development, endorsed by the OECD.

Here we investigated the viability of coconut husk as an alternative to peat in formulating artificial sediments for toxicity testing.

Juvenile Stage IV American lobsters (*Homarus americanus*) were exposed for 15 days in aerated 1L glass jars with 50 grams of test sediment (n = 6 per treatment). Test sediment was prepared with either peat or coconut husk as source of OM. Three OM levels were tested: 0.5, 2.3, and 5 % of sediment wet weight. Dry sediment was mixed with an antiparasitic drug, ivermectin (stock solution in acetone), to achieve 3 desired concentrations (nominal 40, 100, 400 ng/g DW).

Mortality assessment was carried out every day and a 15-day LC₅₀ was calculated with measured concentrations of the compound tested. The test was replicated twice over two years.

There was no statistically significant difference in LC₅₀s between the sediment prepared with peat or coconut husk.

The results indicate that the presence of coconut husk does not introduce toxicity effects of the tested chemicals, suggesting its suitability as a viable alternative for toxicity testing.

2.08.P-Mo168 Tubifex tubifex Bioturbation and Biotransformation Ability Determine the Distribution of Polycyclic Aromatic Hydrocarbons and Polychlorinated Biphenyls Mixtures in the Sediment.

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Bioturbation, the reworking of sediments by animals, plays a significant role in the distribution of contaminants in aquatic environments. The oligochaete *Tubifex tubifex* is known for its sediment reworking and flushing capabilities, which may reintroduce sediment-associated contaminants into the overlying water, as well as influence the bioavailability of these contaminants. Furthermore, *T. tubifex*

may biotransform organic contaminants, thus, impacting the fate of sediment-associated contaminants. This study assessed the impact of *T. tubifex* bioturbation on the distribution of two organic and hydrophobic contaminant groups, namely Polycyclic Aromatic Hydrocarbons (PAHs; considered biotransformable) and Polychlorinated Biphenyls (PCBs; considered inert to biotransformation) mixtures in sediment and their release in the water.

Two experiments (expt. 1: PAHs, expt. 2: PCB mixture) were conducted: Both experiments included a control (i.e., uncontaminated natural sediment) with and without worms and spiked sediment with and without worms ($n = 3$ replicates, 4 worms per replicate). A polyethylene (PE) passive sampler was suspended in the overlying water in each replicate vial to assess the potential distribution of sediment-associated contaminants between the sediment and the overlying water. Contaminants were quantified in the sediments, worms, and passive sampler to determine their distribution and fate in the system. The results provide insight into whether worm presence (bioturbation, biotransformation) created by the behavioral traits of *T. tubifex*, influences the distribution of sediment-associated contaminants, potentially altering their bioavailability in different compartments. This study's findings could advance our understanding of contaminant dynamics in aquatic systems and inform risk assessments and remediation strategies for polluted sediments.

2.08.P-Mo169 The Amphipod *Hyalella curvispina* as an Ecotoxicological Biotool for Environmental Risk Assessment of Sediments in Río de la Plata Ports, Buenos Aires, Argentina

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In this study, samples were collected from port areas of the Río de la Plata near the city of Buenos Aires. A native amphipod was used to evaluate ecotoxicity.

The sampling area covers zones for loading and unloading petroleum derivatives, as well as port activities related to the shipbuilding industry.

Previous studies on age sensitivity in sediments and reference toxicants were made.

Survival and growth curves were analyzed using individuals of *Hyalella curvispina* less than 7 days old and measuring 1.5 mm, exposed to the sediments for 10-15 days. The slopes of these growth curves were evaluated in relation to the characterization and petroleum hydrocarbon content of the samples.

Principal component analysis was used to map the port area based on its activity, defining buffer zones with lower impact. These zones will support future dredging strategies and the management of river sediments near the ports.

2.08.P-Mo170 Assessment of the Detergent Concentrations in Water and Sediments of the Papaloapan River, Veracruz, Mexico

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The Papaloapan River is a system that receives a continuous contribution of toxic compounds due to domestic, industrial, agricultural and livestock activities that take place on its banks. This study evaluated the presence of detergents in water and sediment samples obtained from the riverbed and associated systems, to detect the areas with the highest degree of pollution. Ten samples were collected in the final part of the river (18° 18' - 18° 47' north and 95° 44' - 95° 51' west) during the dry and rainy seasons for 7 years (2014, 2015, 2016, 2018, 2019, 2022 and 2023). The following parameters were evaluated in situ from the water samples: pH, conductivity, dissolved oxygen and temperature. In the sediments, ammonium, organic matter, conductivity, texture, and pH were evaluated in the laboratory. The evaluation of detergents was carried out using the SAAM technique. The concentrations of detergents in the water samples varied from 0.17 ± 0.072 to 0.438 ± 0.2 mg/L. In sediments, from 0.22 ± 0.088 to 0.736 ± 0.438 mg/L. The sediments with the highest concentration of detergents were obtained from areas close to urban areas and areas with low water circulation. Likewise, a higher concentration of detergents will be monitored in 2022 and 2023.

2.09 Aquatic and Terrestrial Plant Ecology, Ecotoxicology and Risk Assessment

2.09.T-01 Temporal Heterogeneity of Periphyton Microbial Activity and Photosynthesis Sensitivity to the Chemical Stress and its Environmental Drivers

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Facing chemical contamination of aquatic ecosystems, it is crucial to better understand how fluctuation in structural and functional microbial biodiversity due to environmental conditions may modulate the sensitivity of microbiomes to chemical stress, as these communities play a significant role in ecosystem function and services. Among these communities, periphytons are complex assemblages of microorganisms that strongly contribute to primary production and biogeochemical cycles. Omics and their combination pave the way to new perspectives in microbial eco(toxi)cology to gain mechanism understanding of the response of microbial communities to their environments. Among others, untargeted metabolomics provides a comprehensive picture of the microbial activity in response to the environment. This approach is complementary of others descriptors at the physiological/functional level that may lack of sensitivity regarding the chemical stress. In this context, the present study aims at characterizing the change of sensitivity to micropollutants of the periphyton at both the molecular and physiological level and unraveling external (water physico-chemistry) and the internal (metabolic and physiological status) factors contributing to such a shift. To do so, biofilms were colonized monthly on glass slides in a pond. At each month, a part of the collected biofilm was exposed during 4h to serial dilution of terbuthylazine (TBA) to assess the sensitivity to this herbicide at both the biochemical (metabolome) and physiological (photosynthetic yield) levels. Our results showed a strong temporal heterogeneity in the sensitivity and response to the chemical stress of periphyton microbial activity (metabolome) against a low one of the photosynthesis. This strengthens the relevance of the aggregated metabolome response to provide an integrative picture of the periphyton sensitivity. This also suggests that quite similar physiological responses may arise from various molecular pathways, adding complexity to the underlying phenomenon. DIABLO analysis further highlighted the contribution of environmental conditions to the temporal dynamic of the basal microbial activity and its sensitivity, although the actual contribution of external vs internal factor in this sensitivity shift remains to be elucidated. Altogether, our results will support better understanding of potential functional impairment of microbiomes by the chemical stress in the global changes context.

2.09.T-02 Investigating the Impact of Herbicide Exposure on Macrophyte Community Composition and Long Term Recovery

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Currently, UK regulatory assessments of risks from herbicides to macrophytes only investigate the immediate, in-season impact of herbicide exposure to individual plants. This study investigates the impact of herbicide exposure on macrophyte community composition and long-term recovery, to understand whether risk assessments should incorporate community level impacts and the ability of individual plants to recover.

Nine mesocosm units were planted with a community of eight emergent macrophyte species in April 2024. Six mesocosms were dosed with metsulfuron-methyl in June 2024. Three of these received 1 µg/L and three received 10 µg/L. Three mesocosms were un-exposed controls. Eight endpoint measurements were recorded for 116 days. These include: Quantum Yield of PSII, length, width, number of leaves, stems and flowers and percent chlorosis and necrosis. Aerial photos of each mesocosm were taken and percent cover calculated in ImageJ. The mesocosms were flushed on day 33 to remove any herbicide. Water samples were collected throughout and analysed by LC-MS/MS to measure the concentration of metsulfuron-methyl.

Principal Response Curve analysis indicates that 93.6% of variation in community level effects between the 10 µg/L mesocosms and the controls can be explained by the treatment. As expected, most species were negatively impacted by herbicide exposure. However, *Phalaris arundinacea* was positively impacted by herbicide exposure, with the population covering a greater percent area in the 10 µg/L mesocosms after exposure compared to the controls.

Some species were able to recover after herbicide exposure. *Myosotis scorpioides* individuals exposed to 10 µg/L initially had a steep decrease in leaf number after exposure. After the flushing event, the number of leaves on these individuals began to increase. At the end of the study the individuals in the 10 µg/L mesocosms had more leaves than those in the controls. Other species, such as *Potamogeton natans*, did not recover after exposure. Individuals of this species exposed to 10 µg/L of metsulfuron-methyl started declining in leaf number 11 days after dosing. By day 74 there were no leaves remaining and these never recovered.

Results suggest that some macrophyte species have the capacity to recover from herbicide exposure, whilst others thrive in communities exposed to herbicide. This highlights the need for risk assessments to incorporate impacts on community composition and their ability to recover.

2.09.T-03 Overspray of Herbicides Poses Risk for Floating Aquatic Macrophytes

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Herbicides from agricultural fields frequently impact edge-of-field aquatic systems via spray drift, drainage, or surface run-off, exposing and affecting aquatic macrophytes. Regulatory risk assessments, especially Tier 1 assessments following OECD guidelines, primarily consider herbicide exposure as occurring via dissolved chemicals in water, a scenario applicable to submerged macrophytes such as *Myriophyllum spicatum*. However, floating and emergent macrophytes, including *Lemna minor* and *Glyceria maxima*, are also subject to direct herbicide exposure via their leaves following deposition of drifting herbicide spray, a pathway not addressed in existing guidelines. Notably, for *Lemna minor*, only a single, limited study from 1989 has explored such overspray exposure, despite recent suggestions of its relevance e.g., in EFSA's recent peer review of glyphosate.

To address this gap, our study compares the effects of glyphosate exposure on *Lemna minor* through two exposure routes: overspray application on the leaf surface and conventional exposure via water. We hypothesized that overspray would result in an increased toxicity compared to exposure via water. Along a dose-response setup, we measured conventional endpoints such as dry weight, and frond number and derived EC50 values for comparison of toxicity between the two exposure routes.

As hypothesized, exposure via spray deposition on top of the leaves led to an increased toxicity across all endpoints measured in *Lemna minor*. Nevertheless, further uncertainties in the method need to be discussed to finally allow drafting a protocol for future standardized overspray testing of floating aquatic macrophytes.

Ultimately, our findings reveal key insights into the differential effects of herbicide exposure pathways on floating macrophytes, with implications for refining regulatory risk assessments and ensuring agricultural practices that better safeguard aquatic ecosystems.

2.09.T-04 Estimating Fifty Percent Effect Rates for Plant Visual Injury via a Decision Tree

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For the authorization of plant protection products (PPP) in the European Union (EU), non-target terrestrial plants (NTTP) are tested for effects on seedling emergence, survival, biomass, growth and visual injuries according to test guidelines (OECD 208 (2006a) and OECD 227 (2006b)). It has to be noted that plant visual injuries (PVI) (chlorosis, necrosis, wilting etc.) are based on subjective and non-standardized observations. Recently, the European Food Safety Authority (EFSA) requires that ER50s for qualitatively assessed PVI should be considered for the risk assessment as equal to endpoints derived from quantitatively determined parameters. However, the interpretation of the data in terms of an ER50 is related to several challenges as it has been discussed previously (Fellman et al; 2023; IEAM).

Furthermore, there is no guidance available harmonizing the assessment method of PVI and the statistical interpretation of the data leading to a multitude of approaches being used. Regulatory Authorities from across the globe have attempted to provide some insight into methods used to evaluate endpoints for differing scoring systems (UBA, EFSA, USEPA, RIVM, HAWC) but no agreement has been reached so far. The PVI SETAC Working Group under the umbrella of the SETAC Plants Interest Group gathered several NTTP PVI data sets to identify the variability and quality of the data. The correct statistical model to be used depends on whether the data is defined as ordinal or continuous. This work aims to harmonize the statistical analysis of PVI data by addressing the various PVI assessment methods used in regulatory NTTP effect studies. It will provide recommendations for effectively analyzing and interpreting the data to enhance comparability with standard ER50s (biomass, survival etc.) in form of a decision tree to support risk assessors. As next steps, the SETAC PVI Working Group will focus on developing a new assessment methodology, a standardized PVI scoring system, which will be accompanied by an appropriate statistical analysis. This progress will require a ring test to investigate the viability of a new approach which requires time. Accordingly, the presented decision tree for choosing suitable statistical methods for PVI evaluation becomes more important as it helps in harmonizing PVI data interpretation as an interim step.

2.09.T-05 First Insights into Drift Capture Efficiency of Non-Target Terrestrial Plants

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Non-Target Terrestrial Plants (NTTPs) are, by definition, located in the off-field where they can unintentionally be exposed to plant protection products. In Europe, spray drift is considered to be the main exposure route for NTTPs and is therefore taken into account for the risk assessment. The extent to which NTTPs are expected to be exposed to drift is defined by drift value tables (Rautmann) which are based on drift field trials using petri dishes as collectors. Drift deposition to flat collectors such as petri dishes is a surrogate for determining spray drift to surface water. However, NTTP communities located in the off-field are 3D structures with variable characteristics such as porosity, size and surface structure (hairy, smooth, waxy, etc.). Accordingly, drift deposition is only covering part of the NTTP exposure and drift capture needs to be considered as well. This raises the questions as to which of the characteristics of the off-field vegetation are most important for determining its drift capture efficiency.

To obtain some first insights into the possible influence that the characteristics of the off-field vegetation have on drift capture efficiency, drift deposition on four species (oats, soya, oil seed rape, sunflower) representing different growth types and 3D structures was investigated with the plants arranged as single plants, rows (1 × 5 potted plants) and blocks (5 × 5 rows) in a wind tunnel. This early work has shown that rows of plants capture essentially the same spray drift as single plants but that blocks of plants differ significantly in the quantity captured. The front row of plants, unsurprisingly, captures the most spray drift whereas just a few rows back, the amount of spray drift captured falls off considerably. There are also differences in spray drift capture depending on plant type.

The results present a challenge to the current *modus operandi* for NTTP risk assessment, in that the standard horizontal collection of spray drift in trials is an oversimplification of the reality of spray drift capture by off-crop canopies.

2.09.P Aquatic and Terrestrial Plant Ecology, Ecotoxicology, and Risk Assessment

2.09.P-We087 Efficacy of *Pseudomonas Fluorescens* and *Azospirillum Brasiliense* Based-Biostimulant in Forage Plant Development in a Historically Contaminated Soil Under Climate Change Scenarios

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Organic farming methods have been increasing in the quest for sustainable and eco-friendly agricultural practices. As an example, microbial biostimulants (mPB) are currently applied in organic agriculture as potential substitutes for chemical fertilizers. Their primary goal is to improve plant growth, mainly by increasing nutrient availability in the soil. However, information on their efficacy under adverse anthropogenic pressure and climate change conditions is limited. This study aimed to evaluate the efficacy of a commercial mPB (comprising *Pseudomonas fluorescens* and *Azospirillum brasiliense*) in a historically contaminated soil with metals/metalloids (e.g., Hg, As, Pb) and under simulated climate change influence. For this, short-term toxicity tests were conducted on two forage plant species the lucerne *Medicago sativa* and the ryegrass *Lolium perenne*. The contaminated soil was collected from an area impacted by industrialization and agriculture (Estarreja, Portugal). The test was conducted under elevated atmospheric carbon dioxide (CO₂) and temperature conditions (800 ppm atm. CO₂ and temperature ramp of 20 30 °C) to simulate a forecasted climate change scenario and control climate conditions (approx. 400-500 ppm atm. CO₂ and 23 °C). For comparison purposes, tests were also conducted with an uncontaminated soil (Lufa 2.2, control) under both climate conditions. Three days after seed germination, the recommended mPB application rate (5 L/ha) was applied to both soils (Estarreja and Lufa 2.2), regardless of the climate conditions tested. After 9 days, soil pH, electrical conductivity, susceptibility profiles of soil bacterial communities to Hg (disk diffusion method), Hg concentration in soils and plants and plant size and biomass were measured. The application of this mPB under distinct climate scenarios did not significantly alter soil pH, electrical conductivity, or mercury levels. *M. sativa* plants grown in Estarreja soil were the only ones showing a significant increase in size and biomass when mPB was applied, regardless of the climate conditions tested. For *L. perenne*, plant size and biomass were similar across both conditions (soil type and climate scenario). This study highlights the positive impact of mPB on plant growth under anthropogenic pressure and adverse climate conditions, especially for *M. sativa*. The observed difference in responses between *M. sativa* and *L. perenne* reinforces the relevance of testing different plant species.

2.09.P-We088 How to Address Herbicide Effects in Lotic Ecosystems – A Test System with Benthic Algal Communities

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In chemical risk assessment, microalgae like *Raphidocelis subcapitata* are tested as representatives for primary producers. However, this planktonic species might be only of little relevance for lotic ecosystems (streams) where mostly benthic algae communities are present. They consist of taxa with different needs regarding environmental conditions and thus also different physiological characteristics. As already shown in several studies for aquatic invertebrates, a similar ecotoxicological response of planktonic and benthic communities and species has not necessarily to be expected.

Thus, to better address the effects of chemical contaminants on benthic algal communities, we developed a test system with natural periphyton that can be exposed under defined laboratory conditions. The tested community develops from a field collected inoculum and contains usually at least 30-60 taxa. So far, diatoms have always dominated in biomass as well as in taxonomic richness both in laboratory culture and in comparative studies in the field. These organisms show, different to planktonic standard species, no exponential growth under laboratory conditions simulating realistic environmental growth. As exponential growth is a pre-requisite for the use of growth rate related figures, the data are evaluated based on biomass related numbers.

During exposure, the development of the periphyton in terms of change in biomass and the composition of the three main algal classes green algae, blue-green algae and diatoms are monitored fluorometrically as chlorophyll-a content. Furthermore, effects on single species are addressed by determination and counting using a microscope at specific timepoints. With an exposure time of up to 14 days, the control variability is low, so that substance effects could be derived well in previous studies. The sensitivity of the investigated biocoenosis differs in part from the results of planktonic single species studies.

2.09.P-We089 Late Nights and Early Mornings; Impacts on Freshwater Algal Morphology

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Freshwater algal species are morphologically very diverse. Among the different taxa they vary in size, shape, colour and structure, making manual identification challenging, thus requiring specialist training. Identification of algal species becomes more challenging during reproduction where algae cells can change in structure and appearance, masking or altering identifiable features. For example, some species release daughter cells, which are miniature clones of themselves, while others surround themselves in a mucilage (palmella stage) in unfavourable conditions and lose identifiable features like flagella. The palmella stage can subsequently become easily confused with species that form colonies contained in a mucilage, like *sphaerocystis Schroeteri*. Reproduction is thought to occur mostly during dawn and dusk in the natural environment, therefore the timing of sampling and preservation of algae samples from the field or mesocosm studies may impact the structure and stages of the algae cells collected. Subsequently this has the potential for misidentification of species collected from the in-life samples. In addition, when conducting mesocosm studies focussed on herbicides, where algae are known to be focal sensitive taxa, the timing of application(s) within these studies may also disrupt these reproductive cycles. Understanding biological processes, such as reproduction, can therefore aid the interpretation of sensitivity observed in specific taxa and help contextualisation of results within mesocosm studies.

In this poster we will investigate how species of algae change appearance in our outdoor mesocosm system during dawn and dusk sampling. With the aim of advancing and improving taxonomic identification techniques and expanding our understanding of potentially sensitive life stages in algae cells.

2.09.P-We090 Combined Effects of Methylmercury and Temperature in *Chlamydomonas reinhardtii*

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Mercury (Hg) is a legacy pollutant that still currently threatens environmental and public health, because of the biomagnification of methyl-Hg (MeHg) in food chains. Microalgae are the major entry pathway of MeHg in food webs. Because environmental and biogeochemical processes are dynamic, understanding the time progression of toxic effects is instrumental for a more in-depth examination of the threat to microalgae fitness in environmentally relevant conditions. This project aims to improve our knowledge on the transfer and toxic effects of MeHg in a warming context using flow cytometry (FCM) to obtain novel insights into the cellular basis underlying the responses to MeHg.

Two incubation temperatures with a 6°C difference based on IPCC projections for 2100, namely 24°C and 30°C, were used. Two exposure conditions were applied for 72h: a control and a 7 ng/L MeHg, corresponding to 1/10 of the maximum MeHg concentration in French continental waters.

In terms of growth, algae incubated at 30°C grew faster regardless of their treatment with MeHg.

Similarly, temperature appeared to play a more significant role than MeHg in altering median levels of chlorophyll autofluorescence and ROS production. Conversely, mortality seemed to be more affected by MeHg treatment, independent of the incubation temperature. Furthermore, temperature did not appear to be a determining factor in the bioaccumulation of Hg. In addition, two cell types emerged in the cytograms of algae incubated at 30°C. Microscopic analysis showed that the algae divided both conventionally and in the form of palmelloids that appeared to secrete more ROS over time. In the ecotoxicology field, dose responses have been more often studied than toxicokinetic, but the former obviously misses some part of the information to assess the physiology and survival of the target biological model over time. Kinetic knowledge is instrumental to better predict effects at higher levels of biological organization and long-term impacts of pollution to propose rational environmental risk assessment in the changing aquatic environment. IFCM was very efficient, as only small volumes are required for the analysis of several endpoints simultaneously making subsampling in the same small vials for all time points and concentrations possible.

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2.09.P-We091 Assessment of the Effects of Metformin on the Macrophytes *Lemna gibba* and *Egeria densa*

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Metformin is a drug used to treat diabetes. It is a drug widely consumed by the population in Mexico and sold without a prescription. Metformin has been detected in wastewater and natural waters at concentrations of 1 to 47 g/L. Because the effects of Metformin on aquatic organisms are not fully known, the objective of this study was to evaluate the effects of this drug on the macrophytes *Lemna gibba* and *Egeria densa* on their growth, their pigment and macromolecule content, and their oxidative effect. A bioassay was performed where the macrophytes were exposed to five concentrations of the drug (10, 20, 30, 40, 50, 100 mg/L), for 96 hours, to determine the EC50 using the Probit method. Subsequently, a 10-day sublethal bioassay was performed, where 3 sublethal concentrations (EC10, EC5 and EC1) were tested to evaluate the content of pigments, macromolecules and their oxidizing effect. The Effective Concentration 50 (EC50) values (96 h) obtained are in the range of 10 mg L⁻¹ 100 mg L⁻¹ indicating that Metformin is a harmful compound for macrophytes (GHS, UN 2011). In the sublethal tests, a decrease of between 15% and 46% in the concentration of total chlorophylls was observed. An increase of up to 80% in the carotene content was detected. The chlorophyll a / chlorophyll b ratio values were lower than those obtained in the controls, indicating that the macrophytes exposed to the drug were in a state of stress. Metformin had harmful effects on macrophytes at concentrations present in aquatic systems.

2.09.P-We092 Effects of Cosmetic Products on Photosynthetic Active Organisms in Freshwater Systems

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The negative impact of sunscreens on marine ecosystems, especially coral reefs, is currently being discussed. One focus is on the effects on the endo-symbiotic algae in corals. However, in addition to the widespread symbiosis with algae, the number of non-symbiotic algae is many times higher. In addition to these microalgae, there are also a large number of macroalgae in the marine environment.

In contrast to marine ecosystems with large amounts of water and a high dilution potential, many freshwater systems, especially lakes, are heavily stressed during the summer months. High concentrations of chemicals were measured, particularly during dry periods.

In fresh water, the function of macroalgae is usually performed by higher aquatic plants. For this reason, the influence of sunscreens on aquatic plants was investigated. We present data collected according to international guidelines. The focus was on the OECD guideline 239 on aquatic plants in a water-sediment system.

2.09.P-We093 The Effect of Elevated Temperature on Pesticide Terbutylazine-induced Changes in Duckweed *Lemna minor* Growth and Oxidative Stress Levels

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Anthropogenic chemical pollution has the potential to pose one of the largest environmental threats to humanity. Water pollution becomes alarming since anthropogenic activity results in the contamination of water bodies with various chemicals, which in turn reduces ecosystem functioning. Pesticides stand out for their widespread occurrence in water bodies and highly diverse biological effects. They can enter adjacent water bodies through different ways and their residues may lead to chronic lethal and sublethal effects in non-target organisms. In the European Union, herbicide terbuthylazine (TBA), a member of the chloro-s-triazine group, has emerged as one of the most frequently detected herbicides in water bodies. However, TBA toxicity to aquatic plants is poorly documented. Climate change is an increasingly urgent problem with potentially far-reaching consequences for life on Earth. There is growing importance to understand the effects of changing environmental factors on pollutants ecotoxicity to aquatic organisms. To address this knowledge gap, we aimed to test the influence of elevated temperature (23° C vs 27° C) on ecotoxicological effects of pesticide terbuthylazine to *Lemna minor* growth and oxidative stress levels. *L. minor* morphological indicators (fresh weight, frond area), biochemical indicators (enzyme activity and metabolites) as well as oxidative stress damage (lipid peroxidation) were measured.

2.09.P-We094 Extraction and Quantification of Caulerpin in Different Caulerpa Species

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Green algae of the genus *Caulerpa* are found in shallow tropical and subtropical marine environments. About 160 species have been reported; two of the most common species are *Caulerpa lentillifera* and *Caulerpa racemosa*, known as sea grapes or green caviar. These species are edible, and their commercialization is an important economic activity in Indo-Pacific regions. There are, however, other species, e.g., *Caulerpa taxifolia* and *Caulerpa cylindracea*, that are considered invasive due to their fast adaptation and rapid expansion that threatens local marine environments. Caulerpin is a secondary metabolite found in the *Caulerpa* species. Concerns have been reported about the risk of the accumulation of *Caulerpa* metabolites, such as caulerpin, in fish tissue and other species of the trophic chain. However, some studies reported that caulerpin caused no harm; specifically, studies on zebrafish have shown increased food intake and improved fish reproductive performance. In addition, in other studies, the antimicrobial and anti-inflammatory properties of caulerpin have been reported, highlighting its bioactivity potential to be yet exploited. However, few studies have focused on extracting and comparing caulerpin content among different *Caulerpa* species. In this work, we present data comparing different extraction methods and quantification of caulerpin in six different *Caulerpa* species.

2.09.P-We095 Identification of Negative Influence of Pharmaceuticals on the Biodiversity of Freshwater Ecosystems with Focus to Water Plant Communities

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The interaction of several species in the ecosystem prevents the risk of biological collapse. Aquatic plants are able to stabilise freshwater ecosystems in a significant way. Further, it is now very evident that climate change is significantly affecting most ecosystems, leading to potentially dramatic changes in food webs. All photosynthetic organisms that can store carbon from the atmosphere therefore have a central role to play. It is therefore important to identify potentially toxic substances on aquatic plants and to clarify the effects under laboratory conditions. We are currently working on the identification of stressors for macrophytes. It is becoming clear that even pharmaceuticals that have not been tested for their effect on rooted macrophytes could reduce plant communities. Here we present data to clarify the impact of pharmaceuticals on aquatic plants.

2.09.P-We096 Comprehensive Analysis of Water Hyacinth Biomass for use as an Agricultural Soil Regenerator

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Water hyacinth (WH; *Eichhornia crassipes*) is one of the most problematic invasive aquatic species worldwide, causing significant ecological and socioeconomic impacts. Its control and minimization of costs associated to its removal may rely on the valorization of WH biomass. Accordingly, this study aimed to characterize WH biomass (morphology and biochemistry) with the aim of incorporating it as an

agricultural enhancer. For this purpose, a multidisciplinary approach was taken, including: (1) sample collection in six strategic locations [Estação Experimental António Teixeira (EEAT), Praia de Coruche (PRAIA), Vila Valente (VV), São João de Loure (SJL), and two points in Pateira de Fermentelos, Aveiro - Observatório Bico (Bico), and Estalagem (PF)], (2) plant biometric measurements, (3) sugar and lipid quantification, (4) pigment extraction and quantification, (5) aqueous extracts conductivity and pH, (6) chemical composition through FTIR; and (7) cellulose, hemicellulose, and glucose levels. The morphometrics and biochemical characteristics of the biomass collected at different locations correlated well with the environmental parameters of each location with plants collected at sites with high input from agricultural activities (EEAT) being larger with a smaller root extension, whereas plants at less urbanized sites were smaller with very dense root systems, which may be related to the availability of nutrients in the water courses. Water extracts pH of each of the biomasses was distinct from those of agricultural zones (EEAT, PRAIA, VV, and SJL, ranging from 6.8 to 8.1) to less urban zones (Bico and PF values of 5.9 and 5.3, respectively), whereas the conductivity values of the WH water extracts were above 6 mS/cm. Water bodies (sampling sites) with a more neutral pH and high conductivity may indicate a higher concentration of dissolved salts and potential contamination from other sources. FTIR analyses indicated the presence of a large area under the absorbance ranges of 2850-3000 cm⁻¹ and 3000-3100 cm⁻¹, indicating the potential presence of aromatic compounds, alcohols, N-H-containing amines or amides, or carboxylic acids. Around 48-63% of the biomass was lignin and hemicelluloses, and glucose levels were above 0.53 µg/g dry weight. Overall, the characterization of WH biomass makes it a promising environmentally friendly resource for agricultural applications through a value-added chain, while designing a sustainable solution to control WH.

2.09.P-We097 Species Sensitivity Distribution for Aquatic Macrophytes: Species and Distribution Selection

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In the EU, a risk assessment for plant protection products (PPP) is required under Regulation (EC) 1107/2009. Referring to the EFSA (2013) aquatic guidance, a tiered risk assessment can be followed where Species Sensitivity Distributions (SSDs) are proposed as a higher tier option for a refined risk assessment. SSDs are crucial tools for deriving the hazardous concentration for 5 % of species (HC5) to predict a regulatory acceptable concentrations (RAC). This can then be compared to modelled or predicted environmental concentrations (PEC) for authorization of PPP. In the current research project, we focus on the selection of macrophytes species and underlying distribution models for constructing SSDs. EFSA (2013) aquatic guidance notes that in SSDs, all species have equal weight and thus are considered of equal importance in assessing the ecotoxicological risks. But what species of aquatic macrophytes should be included in the SSD? With this question in mind, we investigate what species are actually possible to test in laboratory conditions to fulfil existing validity criteria of OECD test guidelines. The analysis is based on accepted studies as evaluated at the EU level during (re-) authorisation of active substances. We look at how and why some species are excluded from constructing an SSD. We also identify which macrophyte species grow in edge-of-field water bodies (ponds, streams and ditches) and which species can be considered as characteristic species. The characteristic species show a large overlap with the species typically used in the SSD approach.

EFSA (2013) aquatic guidance also states that While it is typically assumed that SSDs follow a normal distribution, significant deviation from normality (..) should be a trigger for trying other distributions, (e.g., Burr type III, Weibull) that may provide a better goodness of fit. Do the users of SSD often check the goodness of fit of the dataset for a particular PPP? We explore the evaluated active substances at the EU level to identify what distribution models have been typically used and whether goodness of fit was verified. We analyze whether other models would fit the datasets better and how the selection of underlying distributions may influence the agreed RAC. In addition, we explore the ready to use tools that allow a goodness of fit check and selection of distributions, e.g., ETX, Seb Dalgarno (2018) ssdtools, UBA R-based excel tool.

2.09.P-We098 Aquatic Macrophyte Toxicity: Water Phase vs Overspray Application

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Following the data requirements (Commission Regulation (EU) 284/2013) for plant protection products with an herbicidal mode of action, effects on emergent (or floating) macrophytes (e.g., *Lemna gibba*) shall be assessed following test guideline OECD 221 (standard aqueous exposure). As most herbicides are applied by spraying, the member states of the Central Zone are of the opinion that (direct) exposure of floating macrophytes by spray drift should be considered as an additional scenario in the risk-assessment

in case of unexpected low toxicity ($\text{ExC}_{50} > 1 \text{ mg/L}$) for the aqueous route.

Spray drift exposure can be recreated by an overspray-design in the laboratory. While this design is nowadays a common experimental procedure for terrestrial ecotox-studies, no guideline or validated test protocols are available to test aquatic macrophytes.

We herein present our developed and established test system to facilitate an overspray exposure scenario for *L. gibba*. Performance criteria have shown to be passed with high repeatability as well as precision and the results of initial investigations with an exemplary commercial herbicide comparing the two modes of exposure are presented.

2.09.P-We099 Comparing the Suitability of Non-Destructive Macrophyte Endpoints

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Endpoint measurements are commonly used to assess the impact of chemical exposure on plant health. However, there is little research focusing on the reliability of endpoint measurements, meaning that commonly used endpoints may not always be an accurate reflection of plant health. In this study we compare the reliability and sensitivity of eight commonly used non-destructive endpoint measurements and suggest improvements to future plant ecotoxicology studies.

Eight emergent macrophyte species were selected for a long-term mesocosm study investigating the impact of $10 \mu\text{g/L}$ of metsulfuron-methyl on aquatic plants and their recovery. Eight endpoint measurements were selected: number of leaves, flowers and stems, length, width, quantum yield (QY) of PSII (a measure of photosynthetic efficiency, measured using a fluorometer) and percent necrosis and chlorosis (both separate and combined). These were recorded weekly for four weeks after dosing and fortnightly for a further fourteen weeks. The mesocosms were flushed on day 33 to remove any herbicide. Water samples were collected throughout and analysed by LC-MS/MS to measure the concentration of metsulfuron-methyl.

Focusing on just one of the species, results from *Myosotis scorpioides* demonstrated that whilst measuring necrosis and chlorosis poses issues with subjectivity, it is sensitive to changes in plant health. For instance, 46 days after exposure the individuals in the treated mesocosms had between 45 and 80% necrosis, significantly ($p < 0.05$) greater than the individuals in the controls (which all had 5% necrosis). However, both the width and the QY of PSII remained statistically similar across time between the control and treated individuals. The latter may be because only the healthiest part of the healthiest leaf was selected, meaning that if a plant only had one healthy leaf remaining, the results from QY of PSII could make the whole individual plant appear healthier than it is. The individuals in the treated mesocosms had significantly ($p < 0.05$) less stems and leaves than the controls 46 days after dosing, however after the chemical exposure was removed they had significantly ($p < 0.05$) more stems and leaves by day 116. Our results suggest that relying on one endpoint measurement alone can provide unreliable results. In order to fully understand how chemical exposure impacts plant health and their ability to recover, multiple endpoints should be used in conjunction with one another.

2.09.P-We100 Approaching a Harmonized Assessment of Phytotoxicity in Aquatic Plants

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Visual injuries (phytotoxicity) in aquatic plants gain an increasing attention in the risk assessment of plant protection products, despite the lack of a clear validated evaluation/assessment scheme. So far mainly qualitative assessments of observed phytotoxicity effects in the registration studies have been performed. The assessed symptoms (if assessed at all) are often difficult to classify in their severity (e.g., minor, major) and prevalence (share of plants affected). Consequently, a statistical evaluation is hardly possible - if at all. In addition, it is not known which effect at which severity may cause the death of the plants or a significant impact on growth. Even if such data are recorded and assessed, comparison of phytotoxic effects between different labs or even different time periods remains difficult due to the use of individual and non-standardized assessment schemes.

Our goal is to develop and propose a harmonized assessment approach for different types of phytotoxicity symptoms in aquatic plants. In a first step we collect all available data from our studies (approximately 550 *Lemna* and 50 *Myriophyllum* studies), to evaluate the data and to find correlations between observed phytotoxic and conventional endpoints. In further steps we plan to propose an assessment scheme and evaluation process for phytotoxicity in different plant species. By performing lab experiments using chemicals covering a range of different modes of action, we aim to evaluate approaches regarding feasibility, reliability and reproducibility and ultimately define our scheme for assessment of phytotoxic

effects in aquatic macrophytes.

In our poster presentation we give an overview of our first results of the evaluation of our available aquatic plant studies based on our approach.

2.09.P-We101 Extending the Standard Lemna Growth Inhibition Test According to OECD 221 with Quantitative Phytotoxicity Assessments

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Authorities frequently request the inclusion of phytotoxicity-related parameters in endpoint derivation for aquatic macrophyte pesticide risk assessment in Europe. However, regulatory documents on phytotoxicity and guidance for scientifically sound assessments are scarce. In fact, assessment is only detailed for non-target-terrestrial plants. For aquatic macrophytes, relevant technical guidelines OECD 221 and OECD 239 from 2006 and 2014 mention phytotoxicity solely as data to be noted. Phytotoxicity assessment in current ecotoxicity studies remains inconsistent and mostly semi-quantitative, leading to evaluator-dependent variability and limiting reliability. Therefore, additional plant specific parameters such as chlorophyll content might be more promising for a quantitative and same time more reliable phytotoxicity evaluation. For this, a proof-of-principle study has been conducted to evaluate the feasibility of phytotoxicity assessment via Chlorophyll-a (hereafter Chl-a) quantification. In a standard study conducted with *Lemna gibba* following OECD 221 guidelines, with an herbicidal formulation containing two active substances, effects on phytotoxicity (i.e., chlorosis and root length) have been detected. These phytotoxicity effects were found at concentrations below the determined endpoints based on growth rate. Subsequently, a further OECD 221 study modified by a method for chlorosis quantification based on Chl-a extraction and spectrophotometric determination has been conducted.

Chl-a measurements indicated effects at lower concentrations than standard chlorosis assessment, making Chl-a inhibition as expected a more sensitive and reliable indicator of treatment-related phytotoxicity than standard visual semi-quantitative chlorosis assessment. However, it was not possible to calculate definitive endpoints needed for the risk assessment (i.e., EC10 or EC50) for Chl-a as the chosen concentration range did not cover the full scope of effects on Chl-a content. In analyzing dose-response pattern, no clear correlation was observed between Chl-a and growth rate or yield inhibition.

In conclusion, Chl-a content analysis has shown potential as a reliable method for assessing phytotoxicity. We, therefore, suggest addressing phytotoxicity with quantifiable methods such as Chl-a content analysis to reduce phytotoxicity related uncertainty in pesticide risk assessment in Europe.

2.09.P-We102 Elevated Temperature and CO₂ Conditions Attenuate the Toxicity of Sulfamethoxazole on Barley Plants at Low Concentrations but Exacerbate It at Higher Levels

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Sulfonamides (SAs) are the oldest and still one of the most widely used antibacterial agents in veterinary medicine, owing to their low cost and relative efficacy against some common bacterial diseases. However, due to poor metabolization in animals, a large portion of the administered amount is excreted, entering the environment through field fertilization with animal manure and slurry. Accumulated in the soil, SAs can be absorbed by plants, posing a potential threat to human health through the food chain. While the use of antibiotics continues to increase, the effects of these pharmaceuticals on plants are still poorly understood, especially under changing climate conditions. The toxicity mechanisms of sulfamethoxazole (SMX) at different concentrations (0, 1, 10, 25, and 50 mg kg⁻¹) on pot-grown barley (*Hordeum vulgare* L.) plants were investigated in this study under ambient and elevated temperature and CO₂ conditions (ATC, 21/14 °C, 400 ppm vs. ETC, 25/18 °C, 800 ppm, respectively). It was found that ETC conditions increased the photosynthetic efficiency and growth of barley and attenuated the toxic effects of SMX at low concentrations (1 mg kg⁻¹) but exacerbated its toxicity at higher levels. The main mechanism by which SMX affected barley's growth was the closure of photosystem II reaction centers, with a rate increasing with increasing concentration, resulting from functional impairment in the activity of oxygen-evolving complex and non-photochemical quenching. This led to a considerably decreased utilization of excitation energy in photochemical reactions, resulting in almost complete growth retardation at 50 mg kg⁻¹ SMX treatment under ETC conditions. Thus, the worrying effects of SMX on crop production could become even more pronounced with the changing climate conditions.

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2.09.P-We103 Ecotoxicological Test Protocol for the Assessment of Reproductive Endpoints in Non-Target Terrestrial Plants under Greenhouse Conditions

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Ecotoxicological testing to assess the effects of plant protection products on non-target terrestrial plants (NTTPs) under greenhouse conditions is conducted according to standard guidelines (e.g., OECD 208 and OECD 227). EFSA's Scientific Opinion of 2014 on the risk assessment of plant protection products (PPP) for non-target terrestrial plants raised awareness of a potential need to assess effects on plant reproduction. Literature reviews and experimental studies evaluated the feasibility and relevance of plant reproductive endpoints. In summary, it can be concluded that (a) assessment of reproductive endpoints in selected plant species is in principle feasible, (b) there is no clear trend that reproductive endpoints are more sensitive than vegetative ones, and (c) cases may occur where reproductive endpoints appear to be more sensitive depending on, e.g., mode of action of a plant protection product and/or test species.

For cases where effects on reproductive endpoints need to be assessed for regulatory purposes, a standard test protocol is required.

A working group within the SETAC Plant Interest Group collected and analyzed relevant and available data and experiences to provide an ecotoxicological test protocol for the assessment of reproductive endpoints in Non-Target Terrestrial Plants under greenhouse conditions.

This poster will provide an overview of the protocol and will highlight the challenges and special considerations such as test duration, plant species and endpoint selection as well as statistical evaluation.

2.09.P-We104 Environmental Risk Assessment of Plant Protection Product Residues on Imported Cut Roses

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In the EU, the use of plant protection products is restricted to approved active substances in authorised products based on EU legislation. In addition, maximum residue levels on food & feed apply to both locally grown and imported crops. This is not the case for imported cut flowers, which must however comply with phytosanitary requirements. High levels of residues, including substances that are not approved in the EU, have been found on imported cut flowers, but thus far, environmental risks from these residues remain unknown.

Waste of cut flowers may be discarded through green waste and then get composted. Through composting, residues from cut flowers may reach the environment. As the soil is the primary receiving compartment of compost, we conducted an environmental risk assessment for soil organisms and bees exposed to compost that contains residues from imported roses. Bees were included as they may be exposed via plants and the residues encountered in our study include many insecticides, some of which are neonicotinoids.

Roses imported from outside the EU were sampled and subjected to chemical analysis using a QuEChERS extraction followed by GC- or LC-MS/MS. Concentrations were then modelled using Bayesian techniques to estimate mean and 95th percentile concentrations on imported roses. These were compared to safe concentrations in soil, which were derived from ecotoxicological endpoints for soil organisms and bees, and from environmental quality standards for soil and surface water. Safety factors used to calculate safe concentrations in soil from endpoints for plants, soil micro-organisms, earthworms, springtails, soil mites and other soil insects were taken from ECHA and RIVM guidance and were affected by data availability. Safe concentrations for bees were calculated using the scenario for sludge application (ECHA guidance) and using safety factors comparable to those for soil organisms. Environmental quality standards for soil were used as such, while environmental quality standards for surface water were recalculated to safe concentrations in soil using equilibrium partitioning. The lowest safe concentration for each substance was used for the risk characterisation, where P95 and mean concentrations in roses were divided by safe concentrations in soil. The resulting risk ratios varied between <1 and nearly 1000,000, indicating a potential risk to soil organisms and/or bees resulting from imported roses in green waste.

2.09.P-We105 Zn-Al-NO₃ Layered Double Hydroxide as Potential Innovative Zn Nanofertilizer: An Integrated Assessment of its Efficacy and Environmental Safety

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The United Nations predicts the world population will reach 11 billion by 2100. Agricultural production must ramp up to cope with the expected increased food demand. Fertilizers, widely used to enhance plant productivity, often cause soil degradation. Thus, developing innovative products with improved efficacy and environmental safety is crucial. Nanotechnology has shown potential for agricultural use as it is expected to optimize agricultural practices while reducing environmental impacts. Among the several classes of nanomaterials, layered double hydroxides (LDHs) have been receiving increasing attention. Still, considering their novelty, more information on their effects on different terrestrial organisms must be available. To overcome this gap, an integrated assessment was conducted, focusing on the evaluation of the effectiveness of Zn-Al-NO₃ LDH as an innovative nanofertilizer to a target species (maize) and safety to non-target species (enchytraeids and collembolans), along with the exposure chemical assessment. Results indicated that soil fertilization with LDH at a recommended dose, aligned with maize nutritional needs, enhanced plant emergence and shoot length compared to a conventional fertilizer (ZnSO₄). While Zn accumulated in maize roots with low translocation to shoots, root morphology remained unaffected. Comparing the release of Zn, ZnSO₄ was more effective in the immediate Zn supply, while LDH controlled the release of Zn throughout time. In addition, the environmental safety of the nanofertilizer was evaluated by assessing its effects on the avoidance behaviour, survival and reproduction of enchytraeids and collembolans. The results showed that both enchytraeids and collembolans could detect the LDH in soil. Comparing both species, enchytraeids were more sensitive to their presence, likely due to organisms different exposure routes. Reproduction was the most sensitive endpoint, followed by avoidance and survival, indicating a potential risk, as the concentrations usually not perceived as harmful might induce a chronic effect, such as affecting reproduction success and the future of the population. The quantification of Zn in the soil pore water showed that LDH underwent changes and released Zn, which could be responsible for the observed effects on soil organisms. Overall, this study showed that Zn-Al-NO₃ LDH has the potential to replace Zn conventional fertilizers since its application rate does not exceed toxicity thresholds.

2.10 Integrative Approaches to Biomonitoring and Wildlife Toxicity: Improving the Assessment of Exposure and Effects of Legacy and Emerging Contaminants.

2.10.T-01 Brown Rat (*Rattus norvegicus*) Metabolic Adaptation to High Rodenticide Usage in the Sewer System of the City Core of Madrid

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The impressive adaptability of the commensal brown rat (*Rattus norvegicus*) metabolism could explain the wide presence of this species in the sewer system of cities as one common urban pest. Anticoagulant rodenticides (ARs) are used for control of rat populations and multiple studies have shown that rat populations can exhibit resistance to ARs associated with nonsynonymous substitutions in the vitamin K epoxide reductase complex subunit 1 (*vkorc1*) gene. However, few studies explore other resistance pathways. The metabolic hypothesis suggests that increasing metabolism (and detoxification processes) in rodents can lead to a reduction of the half-life of rodenticides in the organism provoking a resistance phenomenon. Therefore, identifying genes associated with this adaptive metabolism could provide valuable information for the complex networks underlying variations in resistance. The aim of this study was to detect the metabolic (including detoxification) pathways involved in rodent resistance by means of RNA sequencing. We explored the transcriptome of rats captured in an area of high rodenticide usage in the sewage system of Madrid. We obtained RNA post-mortem samples of rat tails with high quality (RIN scores >8) for total RNA sequencing library preparations. In total, we obtained 57-79 million high-quality paired reads per sample with a mean alignment rate to reference of 58.2%, after discarding a sample due to low alignment rate (<15%). Samples were stratified according to the number of SNPs and normalized counts of the *vkorc1* gene to define a threshold to group samples into SNP (SNP>5, ncounts>400) and noSNP (SNP < 5, ncounts < 400) groups for differential expression analysis. The number of differentially expressed genes (DEGs) detected ranged from 5,359 to 7,106 depending on analysis, of which 4,081 were common to all analysis performed. From the Salmon-based DEGs, 557 also presented SNPs in their coding sequence. These SNP presenting DEGs were used for Gene-Set Enrichment Analysis (GSEA) on KEGG pathways, showing 6 positively and 18 negatively related enriched categories. Thus, the SNP group had higher expression of the complement and coagulation cascades and lower expression of

xenobiotic, drug or retinol metabolism related genes. Integrated interpretation of both total SNP and differential gene expression will elucidate Adverse Outcome Pathways related with metabolic resistance in rodent populations adapted to a high rodenticide use.

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2.10.T-02 Dermal Pesticide Exposure of Sand Martins (*Riparia riparia*) and Barn Swallows (*Hirundo rustica*) as a Potential Cause for their Acute Death

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The current study investigates two spontaneous mass mortality incidents in two different swallow bird species in the Netherlands. The first incident was observed in a sand martin (*Riparia riparia*) colony near Utrecht and occurred during bird ringing. More than 30 individuals showed direct mortality, plus an additional of 15 individuals that died soon after being caught. The affected sand martins exhibited remarkable symptoms of neurological behavior: proptosis, hypotonia and ataxia. A second comparable case was observed in barn swallows (*Hirundo rustica*) in Noord-Holland. To determine the potential cause of death for these two cases, dead swallows were collected, necropsied and samples were taken for a virological, pathological and pesticide exposure assessment. The virological and pathological examination is work in progress, but the 650 pesticides screening of brain, feather, liver and stomach samples revealed the ubiquitous presence of the pyrethroid insecticides permethrin and tetramethrin. Only traces were detected for some other pesticides, but most pesticides were not detected. The average concentration of permethrin and tetramethrin in barn swallow feathers was 18.6 ug/g feathers (0.8 to 46 ug/g) and 0.7 ug/g (0.05 to 1.5 ug/g) feathers, respectively. The average concentration of permethrin and tetramethrin in sand martin feathers was 62.7 ug/g (11.7 to 137.3 ug/g) and 8.7 ug/g (1.9 to 19.9 ug/g) feathers, respectively. Remarkably, permethrin and tetramethrin concentrations were absent or detected at relatively low levels in liver and stomach samples. An unexpected result based on the available toxicity data for these compounds and the birds remarkable acute neurotoxicological symptoms. These observations suggest that the main route of exposure may not have been oral, but via acute dermal pesticide exposure. A suggestion that is supported by the observed high concentrations of permethrin and tetramethrin in feather samples, and its presence in some brain samples. This finding could potentially help to explain their neurological behavior (work in progress, will elaborate on this more during presentation). Concerning is that current environmental risk assessment of chemicals does often not adequately consider the impact of direct pesticide exposure to wildlife, and knowledge on potential effects of this exposure route is lacking. However, the results of the current study suggest that dermal exposure is the exposure route of relevance.

2.10.T-03 Developing Non-invasive Tools for Monitoring Pharmaceutical Exposure in Avian Scavengers

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Pharmaceuticals are now a well-known environmental concern. Veterinary drugs can reach avian scavengers through the trophic chain when consuming previously treated livestock with potential negative consequences. The most dramatic example of this was the devastating effect that had diclofenac, a non-steroidal antiinflammatory drug (NSAID) in Gyps vultures from Asia. Residues of this NSAID in the carcasses easily reached vultures resulting in an acute renal failure and extended visceral gout. Other NSAIDs have also been identified as highly toxic to avian scavengers, such as flunixin, ketoprofen, nimesulide or carprofen. NSAID intoxications with diclofenac and flunixin have already been detected in Spain, which hosts the biggest European vulture population. Several studies have confirmed the presence of NSAIDs and other pharmaceuticals such as antibiotics, antiparasitic drugs or barbiturates in avian scavengers and livestock carcasses from Spain. Even though, these methods require strong sampling efforts. Therefore, here we developed a non-invasive method to monitor these compounds using pellets as a matrix to collect information on exposure to pharmaceuticals in sites surrounding vulture feeding stations. For this we sampled pellets (n = 292) from vulture roosting sites among different regions in Spain during 2021, 2022 and 2024. We analysed 49 compounds that included 13 NSAIDs, 32 antibiotics, 3 antiparasitic drugs, and 1 anticholinergic drug, using a liquid chromatography coupled with electrospray

ionization mass spectrometry (LC-ESI-MS/MS). Additionally, a subsample was taken from each pellet for identification of the main prey to study their association with the presence of pharmaceuticals. Veterinary pharmaceuticals were detected in 44.2% of pellets: 43.5% with antibiotics, 2% with NSAIDs and 1.4% with both. Regarding the diet, pellets containing only domestic livestock hair showed the highest prevalence (49.5%) for pharmaceuticals, followed by mixed pellets (38.6%), and pellets of wild animals (33.3%). Our results state that vultures in Spain are exposed to a wide variety of pharmaceuticals. We found that certain levels of compounds are influenced by the type of livestock ingested previously by the scavengers such as pig and chicken. Therefore, we emphasize that the disposal of carcasses for avian scavenger feeding must be closely monitored and consider the potential risks posed by veterinary pharmaceutical residues, notably NSAIDs.

2.10.T-04 A Bat-Barber for Metal Exposure?

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Bats represent a fundamental part of the Costa Rican ecosystem, characterized by varied diets and food sources. They are also susceptible to numerous exogenous stressors, making them critical environmental indicators. Collecting Chiroptera biological samples can be a valuable tool for better understanding bat populations' health status and the environment's ecotoxicological condition. Sampling from wild bats can be complex due to their size and ecological characteristics. Furthermore, the less invasive method of collection is required to minimize stress and potential physical damage to the subjects. The value of hair as a reliable sample for metal detection and quantification is well documented in the literature. Despite this, little research on trace elements in bat hair has been conducted in Costa Rica. In this study, we analyzed trace element concentration in the hair of 23 different bat species (320 animals in total) from Costa Rica in the geographic area of Sarapiquí, including frugivorous, nectarivorous, insectivorous, and hematophagous bat species. Bats' hair was collected by capturing subjects using 8 mist nets. Each bat was taxonomically identified on external physiologic characters and shorn in the lumbosacral portion of the back to collect hair using ordinary scissors, and then bats were released. Trace elements (As, Cd, Cr, Hg, Ni, Pb, Co, Cu, Fe, Mn, Se, and Zn) were analyzed using an Inductively Coupled Plasma-Optic Emission Spectrometry (ICP OES) technique on a Perkin Elmer Optima 2100 DV couple with a CETAC U5000AT+ ultrasound nebulizer for mercury. The distribution of heavy metals in bats highlighted no particular differences in the accumulation among species, while differences were found among sampling sites. Cd, Cr, Pb, and As were among the metals showing the highest concentrations, and PCA analysis also revealed that the analyzed elements form, on the totality of the samples, into two groups, one represented by Zn, Ni, Pb, and Hg, the second by the remaining elements. This allows us to infer that diet is not the primary source of metals but rather a deposition at the environmental level, and anthropogenic activities cause the difference in the latter at the various sites sampled. The obtained results prove the effectiveness of hair for non-invasive monitoring of contaminants and the importance of collecting and integrating all possible data in the analysis and interpretation of research outcomes to get to more reliable conclusions.

2.10.P-We115 Monitoring Chemicals Exposure and Mixture Effects in Terrestrial Food Chains in Europe

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We have insufficient knowledge of exposure of terrestrial biota to anthropogenic chemicals in the environment, and of effects on terrestrial biodiversity and ecosystem services. TerraChem is carrying out research on monitoring exposure and mixture effects in wildlife food chains in order to: (1) detect and determine chemicals, and predominant chemical mixtures, in selected apex species food chains in

representative terrestrial ecosystems across Europe; (2) better understand exposure including routes and extent of trophic transfer; (3) explore patterns for individual contaminants and predominant mixtures in the selected food chains; (4) elucidate toxic effects of chemical mixtures on vertebrates; and (5) collate secondary data on contaminants along trophic chains in terrestrial ecosystems in Europe. We implemented a pan-European case study (barn owl *Tyto alba* food chain in 6 countries) and six single-country case studies on apex mammal species food chains. In each country, for each case study, we gathered samples from six apex species territories. We gathered one apex species liver sample per territory. Four samples of each type of food chain sample were pooled to give, for each territory: 1 pooled vertebrate prey species A (e.g., wood mouse *A. sylvaticus*) soft body tissues sample; 1 pooled vertebrate prey species B (e.g., field vole *M. agrestis*) soft body tissues sample; 1 mixed invertebrate species group A (herbivore/fungivore/detritivore) sample; 1 mixed invertebrate species group B (omnivore/carnivores) sample; 1 mixed plant species leaves sample; 1 bulk soil sample. For each territory, food chain samples were collected within a short time-frame for temporal co-location. Samples will be analysed using: (1) instrumental analysis with ultra-high performance LC-HRMS and GC-HRMS for identification of c. 2,500 known emerging substances and suspect screening of >100,000 substances; (2) sensitive targeted methodologies for the determination of legacy substances. Additional analyses will include: (1) CALUX bioassays for analysis of mixture effects; (2) stable isotope analysis to inform analysis of routes of transfer and trophic magnification; (3) metabarcoding of soil and invertebrate samples to support source-to-damage modelling and work on prevention and mitigation of risks. The presentation will include discussion and conclusions on the design of a sampling regime and preliminary results from the target analyses and non-target screening.

Disclaimer/Disclosure: This research is funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or of the Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

2.10.P-We119 Legacy and Emerging Poly- and Perfluoroalkyl Substances (PFAS), and Non-Target Screening in Marine Mammals Stranded around the UK

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Poly- and perfluoroalkyl substances (PFAS) are a large group of chemicals that display unique properties due to combined oil and water repellence, extreme stability, and hence are used in a wide range of industrial processes and consumer products. These chemicals can be problematic in the environment due to their potential to undergo long-range transport, persistence and toxicity. However, only some of these substances have been regulated by the Stockholm Convention and other international regulations. Beyond these known PFAS, numerous other related chemicals remain undetected due to the limitations of traditional targeted analyses. Non-targeted screening allows for the identification of both known and previously unrecognized contaminants.

We analysed the liver tissue of 200 individuals from a variety of species stranded between 2012 and 2022, collected by the UK Cetacean Strandings Investigation Programme (CSIP). We employed an ultra-performance liquid chromatograph coupled to a high-resolution mass spectrometer (Orbitrap) to analyse a range of both legacy and emerging PFAS including 11 PFCAs (C4-C14), 9 PFASs (C4-C10), 3 FASAs, 3 PFPiAs and 6 fluorotelomer acids, among others in mammal liver tissue. Further, we ran pooled extracts using data-dependent acquisition to generate MS2 spectra. CompoundDiscoverer software was used to identify chemicals present.

We report high total PFAS concentrations in a range of species of marine mammals collected around the UK. Concentrations of different compounds vary highly within species, but most species tend to be dominated by PFOS and long chain PFCAs. Total PFAS concentrations had an average of ~ 200 ng/g ww between species, with bottlenose dolphins having the highest total PFAS concentrations. Total PFAS concentrations decreased in our study period (2012 and 2022) for both cetaceans and seals which could be as a result of global restrictions such as the Stockholm Convention. Preliminary results from non-target screening in this study have revealed the presence of a range of active pharmaceutical ingredients and potentially narcotic substances. This study highlights the importance of continued biomonitoring and underscores the complementary use of targeted and non-target screening approaches.

2.10.P-We136 Metal Contamination and Ecological Niche Variations in Sea Turtles of São Tomé and Príncipe

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The São Tomé and Príncipe archipelago hosts nesting sites for four of the seven existing and threatened sea turtle species. Due to their wide distribution, long lifespan, and diverse feeding behaviours, sea turtles often accumulate pollutants, such as metals and metalloids, throughout their complex life cycle. This study investigated metal contamination and isotopic signature in three sea turtle species *Chelonia mydas*, *Eretmochelys imbricata*, and *Lepidochelys olivacea* comparing two different life stages (nesting females, males, and juveniles) from São Tomé Island. Additionally, nesting females green (*C. mydas*) and hawksbill (*E. imbricata*) sea turtles from Príncipe Island were analysed to compare contamination levels and isotopic niches between the two islands. Female olive ridley turtles (*L. olivacea*), which nest exclusively on São Tomé Island, showed higher levels of several elements (e.g., Ca, Fe, K, Zn, Se, Cd), which could reflect natural physiological levels, contamination and/or unique dietary sources. Additionally, green and hawksbill sea turtle populations, including both adults and juveniles, showed elevated concentrations of elements such as S, Cs, and Pb. Moreover, differences in chemical elements contamination were observed between female green turtles across the two islands, despite their similar isotopic signatures. On São Tomé Island, isotopic niche analysis revealed variations in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, suggesting that green, hawksbill and olive ridley turtle populations differ in their ecological niches and/or exhibit dietary habitats, both across species and between developmental stages. This study provides insights into chemical element contamination and ecological niches across sea turtle species in the archipelago, highlighting species-specific and life-stage variations that reflect differences in diet, ecology, and environmental contamination. Furthermore, these findings enhance understanding of contamination dynamics, providing insights that can help local NGOs design targeted conservation strategies and inform policy decisions.

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2.10.P Integrative Approaches to Biomonitoring and Wildlife Toxicity: Improving the Assessment of Exposure and Effects of Legacy and Emerging Contaminants

2.10.P-We106 Chemical Profiling of Hydrophobic Organic Compounds in Terrestrial Mammals at High Trophic Levels Using Silicone Chemometers

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Passive equilibrium sampling using silicone chemometers is an effective tool for sampling and assessing hydrophobic organic compounds (HOCs) in biota. Silicone chemometers have been applied to evaluate HOCs in lipid tissues of various marine mammal species; however, their use for the bioaccumulation assessment of pollutants in terrestrial mammals remains largely underexplored. Here, we address this gap by characterizing the chemical exposome of four terrestrial and two semi-terrestrial mammal species, representing different feeding habits and habitats.

We used silicone chemometers to extract and analyze approximately 100 legacy and emerging HOCs from 83 lipid-rich blubber samples collected from nutria ($n = 10$), otter ($n = 10$), fox ($n = 3$), raccoon dog ($n = 20$), raccoon ($n = 10$), and wolf ($n = 30$). Targeted chemical analysis was performed using gas chromatography coupled with high-resolution mass spectrometry (GC-HRMS Orbitrap Q-Exactive), covering polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), chlorinated hydrocarbons (CHCs), and organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), pyrethroids, musks and other industrial compounds. Here we focus on PBDEs, PCBs, OCPs, and CHCs, as data evaluation for the other HOCs is ongoing.

We identified 19 out of 37 target compounds in at least one of the 83 blubber samples, while 18 were absent from all samples. PCBs and OCPs were the most frequently detected HOCs across all species. Furthermore, PCB153, PCB170 and PCB180 were present at elevated concentrations compared to other PCBs. The metabolite 4,4'-Dichlorodiphenyldichloroethylene (DDE) was determined in all otter samples (up to 119 pg/mgPDMS), half of the raccoons (up to 36 pg/mgPDMS), and 12 wolves (up to 22 pg/mgPDMS) but was absent in nutria. The presence of DDE indicates historical contamination with 4,4'-Dichlorodiphenyltrichloroethane (DDT), with DDE being detected five times more frequently than its precursor. Higher concentrations and detection frequencies of HOCs in otters than in other species investigated in our study suggest distinct exposure through their diet and habitat.

Overall, our findings highlight the persistence and bioaccumulation of legacy pollutants, such as PCBs and DDE, in high trophic level terrestrial mammals, despite regulatory bans on these HOCs. Future work will expand the range of HOCs and explore patterns across terrestrial mammals, marine mammals, and humans in our HGF project EXPOSOMETER.

2.10.P-We107 Enhancing the Risk Assessment of Pesticides on Bats: A Case Study from the SYBERAC Horizon Project

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Bats are an order of mammals that might be especially vulnerable to contaminants due to their unique characteristics as high metabolic rate and K -selected reproduction. In addition, they are particularly susceptible to dermal exposure because of their large and highly vascularized wing surface.

Since bats inhabit and forage in farmlands, they are at risk to pesticide exposure, nevertheless, recent research questions if bats are adequately covered by the current European Food and Safety Authority (EFSA) guidance for risk assessment for birds and mammals from plant protection products (PPP). On the other hand, this guidance excludes rice fields scenarios due to its cultivation in water. This research aims to investigate the exposure to pesticides of the bat species *Pipistrellus pygmaeus* and *Pipistrellus nathusii* foraging in the rice fields of Ebro Delta (Spain), and the direct and indirect effects that they might pose.

In the present study, nearly 60 bat boxes are included, placed near rice fields with an ascendent gradient of synthetic pesticide usage: from 0, in organic fields, up to 14 active substances along the rice growing season.

To estimate the exposure to contaminants, samples of blood and faeces of bats as well as samples of arthropods will be collected along the rice growing season and analysed by LC-(HR) MS/MS.

In order to assess the potential direct effects at an apical level, the corporal condition of each specimen sampled will be determined. Bats behaviour will be studied by analysing the differences in foraging activity, which will be quantified with ultrasonic recording devices. In addition, reproductive success will be investigated. The indirect effects will be assessed by studying the abundance and diversity of arthropods that constitute bat s food supply. The characteristics of the landscape surrounding the rice fields will be taken into account, as presence and quality of field margins or the proximity to natural areas. This study will provide necessary information about exposure and sensitivity of bats to pesticides, helping to conduct an improved Environmental Risk Assessment (ERA) for bats in agricultural environments. Ultimately, it will assist in proposing measures towards the alienation of rice production and biodiversity conservation.

This research is one of the study cases of the SYBERAC Horizon Project, which aims to better understand the chemical impacts on biodiversity and ecosystem services.

2.10.P-We108 Immunoglobulin G Characterisation in Iberian Hare Inhabiting Agricultural Landscapes and Exposed to an Infectious Disease

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During the 20th century agricultural landscapes have undergone major alterations due to agricultural intensification, which has been linked to population decline of plants, arthropods, birds and mammals. Agricultural intensification has led to a significant homogenisation of the landscape due to land consolidation, and to the entry of more and more chemical pesticides and fertilisers into the environment. Pesticides directly and indirectly destroy many target organisms for which they were designed, altering the food chains in natural ecosystems. But they also have lethal and sub-lethal effects on non-target organisms. Sub-lethal effects are related to chronic exposures and can have consequences in the health status of individuals, being able to affect the immune system. The immune system is particularly relevant to protect organisms against pathogens such as those that cause infectious diseases. A good study model of this relationship is found in the lagomorphs of the Iberian Peninsula, as the Iberian hare (*Lepus granatensis*) and the effect of myxoma virus (MYXV, genus *Leporipoxvirus*, family *Poxviridae*). For that, blood samples were collected from hunted animals during the 2021/22 hunting season in the southwestern Spain from animals with and without lesions compatible with myxomatosis. For this study the levels of immunoglobulin G, together with MYXV antibodies in serum, and viral presence, were analysed for a first immune system characterisation in animals from pesticides-treated and untreated areas. Overall, the results show that hares from treated areas have lower mean IgG concentrations than those from untreated areas. In addition, the results show particularly low IgG concentrations in animals with lesions, viral presence, and absence of antibodies, suggesting that this disease may be occurring more severely in individuals living in areas treated with pesticides.

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2.10.P-We109 Assessment of Environmental Contamination and Pathogens in the Conservation of Bats in Wetland National Parks in Spain – QUIROTOXPATH Project

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Bats provide essential services for the balance of ecosystems, contributing greatly to their health. However, the scarcity of research on this group means that there is still a significant lack of knowledge about the current status of the populations for most species, making it difficult to prioritise and plan actions for their conservation. This project aims to address, from a multidisciplinary approach, the repercussions of exposure to environmental pollutants and the circulation of pathogens, both individually and synergistically, on the conservation of bats in wetland National Parks (Doñana and Tablas de Daimiel), characterised by being surrounded by large areas of intensive cultivation where the use of pesticides is abundant. In this context, the proposed objectives are: (1) to determine the level of exposure to environmental pollutants; (2) to identify and characterise the groups of pathogens present in the populations, as well as the ectoparasites they carry; (3) to determine the potential effects of oxidative stress and genotoxic effects triggered by environmental pollutants; (4) to determine the immunological status of the populations; and finally (5) to assess the potential association between exposure to environmental pollution and the presence of pathogens/ectoparasites. The results of the project will serve as a basis for the sustainable management of chiropteran populations and contribute to their conservation.

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2.10.P-We110 PharmaBat: Investigating Bat-Mediated Transfer of Aquatic Pharmaceuticals to Terrestrial Ecosystems and Implications for Bat Health

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Pharmaceutical pollution poses a growing threat to wildlife, with traces of medicines detected in nearly all parts of the world. While much research has focused on the impact of pharmaceuticals on aquatic organisms, their transfer to terrestrial ecosystems remains largely unexplored. Insectivorous species like bats, which feed in areas bordering water bodies, could be key players in moving these contaminants from water to land. This pollution can harm bats' health, potentially affecting their immune system, stress response, and ability to transmit diseases. Given bats' role as carriers of various pathogens, some of which can be transmitted to humans, understanding how pharmaceutical pollution affects these species is crucial. PharmaBat is a global-scale project that aims to shed light on the impacts of pharmaceuticals on bats and their ecosystems using an integrative approach. Specifically, we are investigating if bats are exposed to pharmaceuticals through their diet, and how the contaminants' load could influence their overall health including the presence of viruses to ultimately link this with their ability to spread infectious diseases. We optimized pharmaceutical quantification using Dried Blood Spot (DBS) a minimally invasive and field-friendly technique. This method has a substantial potential to advance the field of wildlife ecotoxicology with numerous applications to evaluate wildlife health and contaminant exposure. Here, we present preliminary results of pharmaceutical contaminants found in riparian bats in Sweden using DBS and highlight the potential for improving pharmaceutical monitoring exposure in wildlife.

2.10.P-We111 Bat Activity in Agricultural Land

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Bats are prevalent across Europe, roosting in trees and buildings, foraging in woodland and agricultural land. They are indicator species of a productive habitat. However, bat populations are falling in many areas, with the use of pesticides one suspect as the cause of these declines. This led, in 2019, to the European Food Safety Authority assessing whether the potential risks to bats from pesticides were currently adequately captured within the current regulatory scheme. Their conclusion: they were not. However, no further progress has been made since on establishing a suitable approach to ensure the protection of bats in the future. The first step in developing such an approach is understanding which species are potentially susceptible to exposure to pesticides. To address this, we have conducted an acoustic monitoring study of bat activity in farmland in Northern England from May 2022 to the present. Bat detectors were deployed for one week every month at five different sites across a single farm. The sites were associated with grassland, woodland (control) and three arable fields (growing winter and spring cereals, oil-seed rape and legumes). The crop growth stages (BBCH) were assessed each month. The data was analysed to explore the bat activity and species diversity associated with each site across the study. Early results show that although bats hibernate, some bats were active during the winter period (though at a much lower level than in the summer months), indicating that potential pesticide exposure may still occur year-round. Furthermore, whilst nine species of bat were detected, certain species dominated the recordings (i.e., *Pipistrellus pipistrellus*, *Pipistrellus pygmaeus*) which may be relevant when considering focal species selection for risk assessment scenarios and potential refinement options (e.g., proportion of time foraging in a given field; equivalent to the PT refinement in the birds and mammals guidance document). Given the potential confounding variables (e.g., temperature, rainfall), further analysis will be performed and presented to account for these and assess whether bat activity / diversity differs with crop type and crop growth stage. These results will then be compared against pesticides commonly used on these crops.

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2.10.P-We112 Hops in Crops: Monitoring Lagomorphs in Agricultural Landscapes

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"Brown hares (*Lepus europaeus*) and European rabbits (*Oryctolagus cuniculus*) commonly occur in agricultural landscapes across Europe and are potentially exposed to pesticides mainly via their herbivorous diet including many crop plants. Both species are regularly discussed as potential focal species for the evaluation of associated risks within the European risk assessment framework for pesticides. Understanding their population dynamics, utilisation of agricultural fields and general movement patterns based on robust data from the field is thus crucial for conducting informed risk assessments. Here we want to present comprehensive approaches for monitoring free ranging lagomorph

populations, with a focus on methods that are particularly relevant for assessing pesticide exposure risk, as outlined in the recently noted revised EFSA Guidance Document on Risk Assessment for Birds and Mammals coming into place in 2025.

We present a combination of direct and indirect monitoring techniques, including but not limited to:

- visual observations using night-vision and thermal imaging cameras from elevated locations for documenting general lagomorph activity
- stationary and moving wildlife and drone-mounted cameras
- options for detailed tracking of individual movement patterns and habitat preferences such as VHF- or GPS-based radio tracking

For rabbits in particular, a critical challenge in monitoring this species in crops lies in the spatial relationship between the location of their burrow and the surrounding agricultural fields, and whether the crop of interest is available at known rabbit sites having the rather small activity ranges of rabbits in mind. The selection of monitoring sites thus requires careful consideration of this species-specific ecological trait.

In line with the Bird and Mammal Guidance Document, which highlights the need for robust and case-specific field data, this overview of methods provides valuable options for assessing ecological data for the use in risk assessments of lagomorphs. By integrating multiple monitoring techniques, the application of the given methods contributes to a better understanding of their potential exposure pathways in agricultural environments, and at the same time to a knowledge gain in farmland ecology of these species. We will present examples of more than 10 years of experience in lagomorph field studies in European farmland.

2.10.P-We113 Linking LIFE APEX Data on Chemicals in Wild Biota in Europe with the Distributed System of Scientific Collections (DiSSCo), for Research and Regulatory Applications

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DiSSCo (The Distributed System of Scientific Collections) is the new world-class Research Infrastructure (RI) for Natural Science Collections. It aims to digitally unify all European natural science assets, in a pan-European digital collection sharing common access, curation, policies and practices across countries, ensuring that all data complies with FAIR and CARE principles. DiSSCo also links data on digital specimens in participating collections with related infrastructures such as the Global Biodiversity Information Facility (GBIF), International Barcode of Life Data System (iBOLD), and European Nucleotide Archive (ENA). The EU-funded LIFE APEX project (2018-2022) analysed chemicals in apex predators and their prey from across Europe using non-target screening (>65,000 substances), wide-scope target analyses (>2300 substances) and other target analyses. The resulting data is stored in the LIFE APEX Knowledgebase which is integrated into the EMPODAT database (target analyses data) and the DFSP database (non-target screening data) on the NORMAN Platform. A similar database, also to be integrated with EMPODAT and DFSP, is under construction by the Horizon Europe project TerraChem (2023-26) which will analyse chemicals in terrestrial food chains across Europe. LIFE APEX and TerraChem will together provide >7.5 M data points on chemicals in wild biota. The EMPODAT and DFSP databases are in this respect well placed to contribute to the proposed EU Common Data Platform for Chemicals. The purpose of this study is to develop and test data pipelines to connect DiSSCo architecture with the LIFE APEX Knowledgebase with a view to extending this to the TerraChem Database and other data on chemicals in biota within EMPODAT and DFSP. Such connections will benefit various end users (e.g., researchers, regulators) for a range of applications e.g., locating matched chemical exposure and species richness data to derive species sensitivity distributions for modelling of chemical effects on species richness. We will develop a suite of user stories that identify potential users and prioritise use cases, align data standards between the LIFE APEX database and DiSSCo's Digital Specimen architecture (DiSSCo DS), and enable DiSSCo users to retrieve data from the LIFE APEX database and DiSSCo DS through APIs. This poster will report on preliminary results of this project including user stories developed, any challenges identified in establishing the link and next steps.

Disclaimer/Disclosure: This work is funded by the European Union. Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

2.10.P-We114 Generating Spatially and Temporally Matched Data at Pan-European Scale on Chemicals Exposure and Species Composition in Soils and Invertebrate Communities to Build Species Sensitivity Distributions

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In order to build species sensitivity distributions (SSDs) to train models on effects of chemicals on terrestrial species composition, we need matched data on (a) exposure to chemicals and (b) species composition, for the same place and time. There is at present very limited availability of such matched data for the terrestrial environment, in particular across trophic levels, and in particular at pan-European scale. This study aimed to generate such matched data for multiple locations across Europe for both soil microbiota and invertebrates and was performed under the Horizon Europe project TerraChem. Co-located soil and invertebrate samples were collected as part of 12 case studies, six on the barn owl *Tyto alba* food chain (in Netherlands, Germany, Spain, Portugal, Greece, Romania - which together form a pan-European case study) and six single-country case studies (stone marten *Martes foina*, Netherlands; grey wolf *Canis lupus*, Germany; European badger *Meles meles*, Spain; Egyptian mongoose *Herpestes ichneumon*, Portugal; northern white-breasted hedgehog *Erinaceus roumanicus*, Greece; red fox *Vulpes vulpes*, Romania). For each case study, we gathered six replicate sample sets, one set within the assumed territory of each apex species specimen (roadkill). Each set comprised: one apex species liver sample, eight mammalian prey species samples (in most cases, rodent soft body tissues), four mixed species invertebrate samples, four mixed species plant leaf samples and four bulk soil samples. For each territory, we derived two pooled prey samples, one pooled herbivorous invertebrate sample, one pooled non-herbivorous invertebrate sample, one pooled plant leaf sample and one pooled soil sample. Lyophilised samples will be analysed using HRMS-based wide-scope target screening (>2,500 substances), suspect screening (>100,000 substances) and additional target analyses (the latter for persistent organic pollutants). Each of the pooled soil samples was analysed by metabarcoding for microbial species composition (bacteria, fungi, eukaryotes) in collaboration with the Dutch national research infrastructure eDentity. Each of the pooled invertebrate samples was analysed by metabarcoding for invertebrate species composition in collaboration with the Dutch national research infrastructure ARISE. Matched data on chemical residues and species composition will be used to build SSDs. The poster will present preliminary results and conclusions.

Disclaimer/Disclosure: This work is funded by the European Union. Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

2.10.P-We115 Monitoring Chemicals Exposure and Mixture Effects in Terrestrial Food Chains in Europe

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We have insufficient knowledge of exposure of terrestrial biota to anthropogenic chemicals in the environment, and of effects on terrestrial biodiversity and ecosystem services. TerraChem is carrying out research on monitoring exposure and mixture effects in wildlife food chains in order to: (1) detect and determine chemicals, and predominant chemical mixtures, in selected apex species food chains in representative terrestrial ecosystems across Europe; (2) better understand exposure including routes and extent of trophic transfer; (3) explore patterns for individual contaminants and predominant mixtures in the selected food chains; (4) elucidate toxic effects of chemical mixtures on vertebrates; and (5) collate secondary data on contaminants along trophic chains in terrestrial ecosystems in Europe. We implemented a pan-European case study (barn owl *Tyto alba* food chain in 6 countries) and six single-country case studies on apex mammal species food chains. In each country, for each case study, we gathered samples

from six apex species territories. We gathered one apex species liver sample per territory. Four samples of each type of food chain sample were pooled to give, for each territory: 1 pooled vertebrate prey species A (e.g., wood mouse *A. sylvaticus*) soft body tissues sample; 1 pooled vertebrate prey species B (e.g., field vole *M. agrestis*) soft body tissues sample; 1 mixed invertebrate species group A (herbivore/fungivore/detritivore) sample; 1 mixed invertebrate species group B (omnivore/carnivores) sample; 1 mixed plant species leaves sample; 1 bulk soil sample. For each territory, food chain samples were collected within a short time-frame for temporal co-location. Samples will be analysed using: (1) instrumental analysis with ultra-high performance LC-HRMS and GC-HRMS for identification of c. 2,500 known emerging substances and suspect screening of >100,000 substances; (2) sensitive targeted methodologies for the determination of legacy substances. Additional analyses will include: (i) CALUX bioassays for analysis of mixture effects; (ii) stable isotope analysis to inform analysis of routes of transfer and trophic magnification; (iii) metabarcoding of soil and invertebrate samples to support source-to-damage modelling and work on prevention and mitigation of risks. The presentation will include discussion and conclusions on the design of a sampling regime and preliminary results from the target analyses and non-target screening.

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2.10.P-We116 Free and Open Source Databases on Terrestrial Pollution of Wild Apex Predators

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Pollutants affect environmental health. As field monitoring/sampling can be cumbersome and costly, linking pollutants to effects on biodiversity may be facilitated by complementing chemical analysis with existing data. As the field is >50 years old, there is a legacy of biomonitoring of wildlife. This poster presents collated and curated existing data on pollutants in terrestrial apex wildlife species. From the literature we aggregated data on organohalogenes like dioxins, PCBs, PDBEs, PAHs, pesticides, heavy metals and metalloids in wild biota. The species covered included Canidae, Strigidae and Tytonidae, Mustelidae, Procyonidae, Erinaceinae, Herpestidae, Ursidae and Suidae. In total, we extracted data from ~1500 papers.

As an example, for metals, we report 20000 values for residue concentrations in 28 different biological matrices, organs and tissues (liver, kidney, bone, brain, etc.). These biomonitoring data on metals cover 32 owl, 18 canid and 22 mustelid/procyonid species in 58 countries, from 1829-2024. We covered a large geographical distribution and contaminant levels to aid modelers and assessors to benchmark risks across time and space. Globally, residue levels in liver, hair, feather, etc., increase with their local levels in soil, but remain subject to many other local factors. We applied rudimentary regression to obtain relationships in time and space and interpreted pollutant abundances by describing data in terms of presumed driving factors, pollutant source, (trophic) niche, geography and background levels.

The data will become open access via open source data management platforms (e.g., NORMAN) and we anticipate may support future risk assessment and modeling. We also anticipate that the work can inform future monitoring of chemical exposure in terrestrial species. Importantly, of the 40 fields for meta-data (unit, matrix, geocoordinates, etc.) about 50% of cells were not filled due to reporting lacunae (e.g., detection limits). As papers screened span varying scientific fields (ecology, analytical chemistry, etc.) and decades, terminology and reporting schemes differ vastly, which complicates data compilation, curation and standardization. This presentation will discuss lessons learned to facilitate data compilation and curation. Lastly, we will use the data to interpret new empirical exposure monitoring in European terrestrial species and food chains (e.g., barn owl, red fox) under the Horizon project TerraChem and related projects.

2.10.P-We117 Monitoring Based Substance Prioritization – Tracing Chemical Pollution Through Apex Predators

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The One Health approach pursued by the European Commission to protect humans, wildlife and ecosystems from the harmful effects of chemicals tries to address the problem of global chemical pollution. Increasing production volumes of a constantly increasing number of unregulated chemicals lead to an urgent need for both improved risk assessment strategies and regulatory action. However, major challenges such as the limited amount of systematically collected effect and emission data, the limited consideration of long-term, mixture and biodiversity effects as well as the absence of field comparison for prospective effect analysis currently limit the available assessment and prioritization tools. Using chemical monitoring data from apex predators, such as marine mammals, terrestrial mammals and raptors, provides a potential breakthrough.

Apex predators enable a long-term assessment of chemical exposure as a consequence of accumulation in food webs. Systematically collected monitoring data could be used in multiple ways to identify and prioritize substances in need of regulation, real-world environmental exposure assessment, field extrapolation or effectiveness evaluation of risk management measures. Despite being explicitly addressed in the REACH regulation and its technical guidance documents, they have hardly been systematically used in the environmental risk assessment so far, e.g., as an additional line of evidence for increased bioaccumulation potential in REACH.

This poster illustrates how chemical monitoring data derived from High-Resolution Mass Spectrometry (HRMS)-based suspect screening analysis in various European apex predator species could be used to identify and prioritize chemicals requiring further regulatory action. The developed decision flowchart combines artificial intelligence (AI)-supported assessments of persistency (P), bioaccumulation (B), mobility (M) and toxicity (T) with cross-checked exposure data, yielding a list of Europe's top apex PBT and PMT substances.

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2.10.P-We118 In Utero Maternal Transfer of Environmental Pollutants in Long-Finned Pilot Whale Mother-Foetus Pairs

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Long finned pilot whales from the North Atlantic are highly exposed to environmental pollutants such as heavy metals (mercury, Hg and cadmium, Cd) and persistent organic pollutants (POPs) like PCBs and PFASs. High levels of these contaminants have been associated with negative effects on bodily functions such as reproduction, the immune system and the central nervous system in marine mammals. Continuous high contaminant loads in the tissues of these animals can thus have severe effects on the pilot whale population. The high contaminant exposure in pilot whales leads to concern for females transferring the chemicals to their offspring during pregnancy and lactation, resulting in the offspring being highly exposed at a vulnerable developmental stage.

Tissues from pilot whale mother-foetus pairs were analysed for heavy metals (Hg and Cd), PCBs and PFASs. Muscle samples were analysed for Hg, liver samples for PFASs, Hg and Cd, blubber samples were analysed for PCBs, and kidney samples for Cd.

The metal analysis showed Hg being transferred to both liver and muscle of the foetus, although lower concentrations were found in the foetus compared to the mother. The Hg concentrations in mothers and foetuses were not correlated in either liver or muscle, but the mother-foetus ratios were negatively correlated with foetus length, reflecting the increased transfer Hg with gestation time. Cd was not detected in foetus kidney or liver, indicating no or very low transfer of Cd from mother to foetus.

PCB levels in foetus blubber were correlated with maternal blubber concentrations when expressed as lipid weight, and levels were less influenced by gestation time.

The PFAS mother-foetus concentration ratios were correlated with foetus length rather than maternal concentrations, indicating an increased contaminant transfer with gestation time. The relative concentrations of PFASs in foetuses varied with the congeners, with some showing higher concentrations in the foetus than in the mother (PFOSA: foetus/mother ratios of 2.6 - 7.3), with the highest relative

concentrations in the largest fetuses.

The results show that the relative maternal transfer of contaminants varies between the different pollutant groups. Especially concerning is the PFAS exposure which appears to be transferred from the mother to the foetus at a high rate.

2.10.P-We119 Legacy and Emerging Poly- and Perfluoroalkyl Substances (PFAS), and Non-Target Screening in Marine Mammals Stranded around the UK

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Poly- and perfluoroalkyl substances (PFAS) are a large group of chemicals that display unique properties due to combined oil and water repellence, extreme stability, and hence are used in a wide range of industrial processes and consumer products. These chemicals can be problematic in the environment due to their potential to undergo long-range transport, persistence and toxicity. However, only some of these substances have been regulated by the Stockholm Convention and other international regulations. Beyond these known PFAS, numerous other related chemicals remain undetected due to the limitations of traditional targeted analyses. Non-targeted screening allows for the identification of both known and previously unrecognized contaminants.

We analysed the liver tissue of 200 individuals from a variety of species stranded between 2012 and 2022, collected by the UK Cetacean Strandings Investigation Programme (CSIP). We employed an ultra-performance liquid chromatograph coupled to a high-resolution mass spectrometer (Orbitrap) to analyse a range of both legacy and emerging PFAS including 11 PFCAs (C4-C14), 9 PFASs (C4-C10), 3 FASAs, 3 PFPiAs and 6 fluorotelomer acids, among others in mammal liver tissue. Further, we ran pooled extracts using data-dependent acquisition to generate MS2 spectra. CompoundDiscoverer software was used to identify chemicals present.

We report high total PFAS concentrations in a range of species of marine mammals collected around the UK. Concentrations of different compounds vary highly within species, but most species tend to be dominated by PFOS and long chain PFCAs. Total PFAS concentrations had an average of ~ 200 ng/g ww between species, with bottlenose dolphins having the highest total PFAS concentrations. Total PFAS concentrations decreased in our study period (2012 and 2022) for both cetaceans and seals which could be as a result of global restrictions such as the Stockholm Convention. Preliminary results from non-target screening in this study have revealed the presence of a range of active pharmaceutical ingredients and potentially narcotic substances. This study highlights the importance of continued biomonitoring and underscores the complementary use of targeted and non-target screening approaches.

2.10.P-We120 Transcriptional Biomarkers of Metal-Induced Stress Contamination in the Critically Endangered Burrnun Dolphin (*Tursiops australis*)

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Marine ecosystems are increasingly threatened by chemical pollutants, including metals, which persist in the environment, bioaccumulate, and pose risks to both marine organisms and human health. In Victoria, Australia, Burrnun dolphins (*Tursiops australis*), a critically endangered and endemic species, are especially susceptible to metal pollution due to their strong site fidelity to regions like Port Phillip Bay and Gippsland Lakes areas heavily impacted by urban, industrial, and agricultural runoff. Notably, these areas are associated with some of the highest recorded mercury (Hg) concentrations globally. Previous studies have shown elevated levels of Hg in deceased Burrnun dolphins, which further underscore the need to investigate the impact of such contamination in live populations. Furthermore, little is known about how metals may be affecting these populations, and looking at their molecular responses, particularly gene expression changes, may be valuable in identifying stress and compromised health.

Thus, this study aimed to investigate metal accumulation in Burrnun dolphins and its potential effects on their molecular stress responses and overall health. By examining the expression of genes involved in key biological processes such as detoxification, antioxidant defenses, immune function, and oxidative stress, the research aimed to identify molecular biomarkers indicative of contamination. Skin biopsy samples were collected from free-ranging individuals using minimally invasive methods. These samples were

preserved in RNAlater and stored at -80°C until RNA extraction and gene expression analysis using quantitative real-time PCR (qPCR). Simultaneously, metal concentrations were quantified using inductively coupled plasma mass spectrometry (ICP-MS). Results showed significant correlations between expression of some genes and metal contaminant levels, pinpointing some candidate genes to be used as biomarkers of interest for biomonitoring campaigns. This study highlights the urgent need for measures to mitigate pollutant exposure in habitats critical to Burrinan dolphin populations and contributed with information and tools that have the potential to be used to advance conservation strategies for safeguarding this species and for the preservation of marine ecosystems affected by metal pollution.

2.10.P-We121 A Dynamic Energy Budget Model for the Wood Mouse (*Apodemus sylvaticus*), an Important Focal Species for ERA

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"Environmental risk assessment (ERA) estimates the risk from pesticide exposure to populations of non-target species based on individual level laboratory data for a test species. This complex task of ERA has traditionally been approached in a simplistic way, with the toxicity exposure ratio (TER) only accounting for differences in (average) daily individual exposure. Five to ten-fold safety factors aim to ensure a low risk of individual and, by extension, population level effects. At higher tiers, field studies are used to provide a more realistic assessment of potential effects, thereby reducing uncertainty, but are resource-intensive and have known limitations.

Various mechanistic methods have been developed to reduce uncertainty in ERA. Population modelling uses dose-response curves and estimated exposure to project effects within a simulated population of the focal species. Dynamic energy budget (DEB) modelling can add realism by simulating the sub-individual processes that lead to growth, maturation and reproduction. Further, a DEB model coupled with a toxicokinetic-toxicodynamic module (DEB-TKTD) can mechanistically simulate individual level toxic effects. A validated DEB-TKTD model can then be used to predict the effects of realistic exposure in the field, differences in species traits can be accounted for by substituting the DEB model for that of a real focal species used in ERA.

High-quality DEB models for focal species can therefore have a clear role in a mechanistic ERA framework. We have developed a DEB model for the wood mouse (*Apodemus sylvaticus*), an important focal species in European ERA (population model also under development). The previous DEB model for the species in the Add my Pet (AmP) library was based on very limited data. Following an extensive literature search by the authors, high-quality data were gathered describing:

2.10.P-We122 Diet Specialization on Earthworms in European Bird and Mammal Species – A scientific Perspective

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Earthworms can represent a significant fraction of the diet of various bird and mammal species. Therefore, their role in mediating the transfer of plant protection products (PPP) through trophic interactions is a critical concern in pesticide risk assessments for birds and mammals in Europe. Earthworms can uptake PPPs from contaminated soil or water, which are then transferred to higher trophic levels through predation. To accurately estimate the risk to birds and mammals through secondary poisoning via consumption of earthworms on treated agricultural fields, it is essential to assess the spatial and temporal factors that influence prey availability. The quantity of earthworms in the diet might change with several species-specific factors. Such factors include: (i) varying degrees of diet specialization, (ii) seasonal variations in diet composition reflecting breeding patterns or (iii) habitat suitability for earthworms mediating prey availability for birds and mammals. So far, no concise reviews are available regarding proportions of, and changes in, earthworms within the diet of bird and mammal species, within the context of PPP risk assessments.

The aim of this study is to identify relevant literature that characterizes the consumption and proportion of earthworm-type diet by European wild bird and mammal species. The focus will be on identifying relevant species as well as accompanying covariates like, time of year, breeding status or habitat of the respective wild bird and mammal species.

A meta-analysis of literature results revealed species-specific differences in the quantity of earthworms in the respective diet, but also a strong seasonality in the consumption of earthworms across species. These results will contribute to identifying relevant species for the risk assessment of secondary poisoning, but also provide additional ecological background regarding the trophic interactions that underlie this risk assessment.

2.10.P-We123 Do We Need an Update on Inhalation Risk Assessment for Wildlife?

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In the European pesticide registration scheme the risk assessment for birds and mammals assumes dietary exposure as main route of exposure to pesticides for wildlife after application in field. However, free ranging animals in farmland can also be exposed to pesticides by inhaling. Volatile agricultural pesticides, such as soil fumigants, are of particular concern, although they are injected into the soil, but can be released into the air during application due to their physico-chemical properties, like high vapour pressure. Known potential effects range from mild and reversible (e.g., eye irritation) to more serious and irreversible effects (e.g., lung necrosis), depending on the fumigant and the level of exposure. Therefore, within the European pesticide registration scheme, an inhalation risk assessment for birds and mammals is required for such substances and becomes a more common case in recent years.

However, there were no recommendations in the current, and even are no recommendation in the new and revised EFSA Bird and Mammal Guideline for pesticides coming into place in 2025 on how to conduct an inhalation risk assessment. Thus, inhalation risk assessments for birds and mammals are still based on the requirements according to an EPPO guideline dated 1994 based on the ratio of the predicted maximum inhalation dose and the relevant inhalation toxicity endpoint. This partly based on physiological allometric data on avian respiratory variables from the 1970s and were already recognised at that time as a preliminary analysis of the relationships between avian respiratory variables and body weight. A revision and reformulation of the procedures, still followed today, is therefore needed. This work provides an update on the subject, considering developments since the 1970s and more recent data. It also reviews the avian and mammalian toxicological inhalation studies and the resulting endpoints and examines the extent to which they can be used for realistic inhalation risk assessment in wild vertebrates.

2.10.P-We124 Evaluating Whether Modern Environmental Contaminants Have the Potential to Cause Eggshell Thinning

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Avian population declines arising from eggshell thinning induced by chemical exposure is now typically regarded as an historical threat. Evidence has started to emerge, however, that a wide range of pollutants are abundant in the environment that could generate eggshell thinning. These include historical legacy pollutants and more novel emergent pollutants. Using a systematic literature review approach we provide an assessment of the potential for six main chemical groups, those being DDT and its metabolites, PCBs, PBDEs, PFASs, NSAIDs and neonicotinoid pesticides, to induce eggshell thinning. Assessment of the quantity and quality of evidence highlight that whilst much additional research is needed a number of these pollutants, in addition to DDT, have potential to induce avian eggshell thinning with potential demographic consequences.

2.10.P-We125 Navigating the Complexity of Bird Life History Traits to Better Evaluate Exposure to Environmental Chemicals

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As part of a constant effort to protect wildlife, risks from exposures to environmental chemicals should be evaluated as accurately as possible. This is a formidable challenge to tackle, considering the number of species involved, and their complex interactions with each other, and with their environment.

Consequently, exposure evaluations must often be simplified, using a series of approximations. In many cases, some assumptions are necessary to compensate for species-specific data gaps, but also to simplify calculations used in a screening approach. Consequently, exposure estimates rarely represent actual field conditions.

More specifically, characterizing exposure of birds to environmental chemicals is particularly challenging given their species diversity, ecological and physiological plasticity, complex seasonal life histories and seasonal niche partitioning, and physiological variations. Part of this complexity is reflected in EFSA's recent regulatory guidance, which accounts for parameters such as foraging guild or habitat preference to refined exposure evaluations in higher tiers of the risk assessment (EFSA 2023). Furthermore, efforts using models that increase realism that include habitat preferences in spatial environments have been shown to result in more realistic exposure estimates that can be verified. However, in both cases, species-specific data such as food and water ingestion rates, food choices, and habitat preference, are important and yet, are rarely available.

To address this issue, and advance knowledge in this space, a HESI Technical Committee is working on establishing a state of the science on the influence of birds' life history traits on chemical exposure. We explore the value of integrating species-specific traits and behaviors, that can modulate chemical exposure and biological responses. We also developed a database of life history traits to identify potential impactful data gaps, and establish trait-dependent commonalities among birds to help more accurately characterize their exposure to various types of contaminants. Based on species vulnerability, we propose a prioritization strategy for life history traits to help determine which ones have the greatest influence on chemical exposure predictions. We provide a qualitative analysis of behavioral and life history parameters most useful in providing greater precision to exposure estimates and highlight areas in need of additional research.

2.10.P-We126 Modeling Chemical Bioaccumulation in Black-Tailed Godwits: Analyze the Extra Risk of Migratory Birds and Identify High-Risk Sites Along Migration Routes

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Animals are increasingly exposed to chemical substances that do not occur in their natural habitats. Such chemicals are continuously introduced into the environment, for example as a result of agricultural and industrial activities. Large predators generally have a high potential for bioaccumulation, as they can ingest large amounts of chemicals with their prey. For large migratory bird species, the risk can even be higher as they need to consume lots of prey as they need to increase their body weight for their migration flights. One of the most prevalent shorebird species is the Black-tailed Godwit (*Limosa limosa*) which migrates to north African countries for the European winter months and forages at different places such as estuaries, rice fields and grasslands during winter or migration. We are developing a model to describe the body weight dynamics of godwit individuals, and the bioaccumulation of chemicals that the godwit consumes with their feed. The combination of body weight dynamics and chemical bioaccumulation allows us to analyze the dynamics of internal concentrations, which can increase during periods of intense energy consumption, such as migration flights, even without ingestion of chemicals. The model is used to integrate data on contaminant exposure and uptake, chemical kinetics, and physiological processes to analyze the accumulation of chemicals in black-tailed godwits across spatial scales. Our aim is to analyze the extra risk migratory bird species may be exposed to, to identify high-risk sites along migration routes, and to understand long-term consequences of contaminant exposure. The research results could serve as a basis for conservation strategies for migratory species, including black-tailed godwits, by establishing a connection between their health condition and environmental quality.

2.10.P-We127 Factors to Consider for Future Assessment of Effects of Pesticides on Avian Reproduction

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Protocols for conducting avian reproduction studies based on the OECD 206 (1984) and OCSP 850.2300 (2012) guidelines typically use 144 adult birds and ~2000 offspring during the test. This poster examines the feasibility of two ways that vertebrate use could be reduced (1) the number of species tested, and (2) by reducing the number of animals within or eliminating a concurrent control group in every test. For the global registration of a pesticide, a reproduction study is required for two gamebird species: an upland species (typically Northern bobwhite, *Colinus virginianus*) and a waterfowl species (Mallard, *Anas platyrhynchos*). In Europe, data in only one species (an upland gamebird) are required but data for a second species (a waterfowl species) are often available as they are generated if a product is also registered in North America, and thus are also considered in the risk assessment. This has led to questions around whether additional protection is being achieved by testing in the mallard vs testing in bobwhite alone. Secondly, there is a growing trend towards virtual control groups in mammalian toxicity testing as a way of reducing vertebrate testing, but to our knowledge this has yet to be explored for birds. In this poster presentation we evaluate and apply learnings from an analysis of Eurofins historical control data as well as the authors experiences working on these studies to provide factors that should be considered for future testing that could help reduce the number of animals used. The questions evaluated will include (1) would the same level of protection be achieved if only one species were tested? (2) could virtual control groups that are constructed from historical control data be used to reduce bird numbers needed for testing? The data presented will subsequently be of interest to regulators, industry and those working on animal alternatives. It is suggested that it may be possible in future avian reproduction study protocols to reduce vertebrate usage per test by mining information from historical control data, but it should be recognized that valuable additional information for the risk assessment is generated by testing in mallard in addition to the Northern bobwhite.

2.10.P-We128 Anticoagulant Rodenticides, Hemorrhages and Fractures in Red Kite (*Milvus milvus*): Nine Years Retrospective Study in France

Philippe Jacques Berny¹ and Irene Valverde², (1)VETAGRO-SUP, France, (2)Vetagro sup, Toxicology, France

Second generation anticoagulant rodenticides (SGARs) have been widely used in France for many years against rodents and voles in agriculture. The Red kite (*Milvus milvus*) is an opportunistic raptor species which makes it vulnerable to poison. The Red kite is resident or partial migrant in France.

A 9-year retrospective study was carried out between the years 2014-2023 in the Laboratory of Toxicology VetAgro Sup (Lyon). The aim of this study is to assess the concentrations of AR in Red kite in France and, their relationship with two main signs found in necropsy: hemorrhages and fractures.

A total of 110 individuals were studied. 74% of the positive analysis to SGARs were received in 4 years (2017, 2020, 2021, 2022). From 2017, individuals with multiple SGARs were detected, and from 2020 these individuals represented more than 50% of the total individuals analyzed. Bromadiolone (n = 42, 32%) and brodifacoum (n = 37, 29%) were the most detected SGARs. Followed by difenacoum (n = 25, 19.2%) and difethialone (n = 23, 18%). Positive analyses to SGARs came from 52 different departments in France, mainly from the Pyrénées-Atlantiques (39%) in the southwest of France.

Positive SGARs individuals with hemorrhages (n = 21, 19%) and fractures (n = 18, 16%) showed median concentrations (min-max) of 0.13 (0.01-0.7) and 0.05 (0.003-0.44) µg/g, respectively. Moreover, a threshold value was calculated to predict the presence of hemorrhages or fractures caused by the presence of SGARs in Red kite. This value may be used as a support tool in the investigation of cause of death in Red kite.

2.10.P-We129 Current Status of Lead Exposure in the Marbled Teal (*Marmaronetta angustirostris*) in El Hondo Natural Park.

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The marbled teal (*Marmaronetta angustirostris*) is one of the most endangered ducks in Spain, as it is exposed to various threats, one of them being contamination caused by lead shot accumulation from hunting activities. In this study, blood samples were taken from 48 marbled teals in El Hondo Natural Park and Clot de Galvany (Alicante) and the concentrations of lead, chromium, manganese, nickel, copper, selenium, arsenic and cadmium were analyzed using ICP-MS. Additionally, 4 animals were tagged with GPS transmitters to evaluate potential exposure sources. No differences in lead concentration were found between the sampling locations, nor sex or weight, despite hunting having been banned in Clot de Galvany for over 2 decades. This could be explained by the insufficiency of the lead ammunition ban, the lack of time after the banning and, above all, the persistence of lead shots in the soil over time. Other results showed higher manganese and nickel concentrations in Clot de Galvany (more urbanized area) and higher selenium and arsenic concentrations in El Hondo (more agricultural area).

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2.10.P-We130 Bioaccumulation of Persistent Organic Contaminants in the Complex Marine, Aquatic and Terrestrial Ecosystem of an Avian Top Predator, the Bald Eagle

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We used the bald eagle (*Haliaeetus leucocephalus*), a top predator, as a sentinel species of exposure and effects of POPs in the Salish Sea region of North America. Blood samples were collected from 91 bald eagle nestlings (n = 64 nests) across eight regions of the Canadian areas of the Salish Sea basin and a reference area on the outer Pacific coast of Vancouver Island. Samples were analyzed for a suite of legacy and emergent contaminants, stable isotopes, and fatty acids. Plasma $\delta^{13}C$, $\delta^{34}S$, and $\delta^{15}N$ values revealed clear spatial patterns reflecting dietary transitions, from marine-derived diets on the outer coast to more terrestrial and freshwater diets in the Thompson River, Fraser Valley, and Fraser delta regions. The isotope data contextualize broad dietary patterns, while the fatty acid ratios provide finer-scale resolution, revealing aquatic versus terrestrial dietary sources. Higher ω -3: ω -6 ratios observed from the outer coast (4.4) compared to the interior river system (0.5) aligned with isotope signatures, reinforcing a marine-dominant diet versus freshwater/terrestrial. Relative compositions of perfluoroalkyl substance (PFAS) were generally similar across regions and were dominated by PFOS (22-84%) and PFUdA (1-32%). PFAS levels were highest in urbanized areas of the Fraser Delta (57 ng/g wet weight) and the South Salish Sea (44 ng/g), associated with proximity to airports, wastewater treatment plants, and

landfills. PFAS were lower in less urbanized areas (15 and 18 ng/g, respectively). Flame retardants (FRs) were uniformly low, with the highest levels observed in Delta (2 ng/g) and the South Salish Sea (3.2 ng/g). Legacy contaminants, including organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), were elevated in the North and South Salish Sea (OCPs: 17 and 15 ng/g; PCBs: 6 and 14 ng/g, respectively), likely due to historical use and some ongoing contaminant release. With the exception of PFAS, all contaminant groups were positively correlated with $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ ($R^2 = 0.2-0.3$), underscoring the role of diet and foraging behavior in driving exposure levels, and the importance of ecological variables in understanding contaminant dynamics within this complex marine to freshwater ecosystem.

2.10.P-We131 Evaluating Pesticide Risks in Rice Fields: A Field Study

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Field studies are essential to assess the effects of pesticides (individually or in mixtures) on populations, communities, and ecosystems under natural conditions. However, this step is still lacking in risk assessment schemes. Post-registration studies are especially urgent in rice fields, a unique and ecologically valuable crop that has received little attention from European regulatory authorities, as shown by the lack of guidance for assessing risks to birds and mammals in these environments.

We conducted our research in the Ebro Delta (Catalonia, Spain), an area dedicated to rice cultivation and an important region for waterbird conservation. Surveys were conducted among farmers to gather information on pesticide use, including the types, quantities, timing, and target pests. This data helped design a sampling strategy involving 35 fields (6 organic and 29 conventional) representing different levels of pesticide use. In these 35 experimental units and 9 natural areas, we conducted biological and physicochemical measurements and collected environmental (water) and biological samples (chironomid larvae, bird blood, and crop and gizzard contents).

Using HPLC-Q-ToF, we identified 24, 22, and 21 compounds in the waters of conventional, ecological, and natural areas, respectively, with bentazone, penoxsulam, azoxystrobin, carboxin, acetamiprid, and chlorantraniliprole at higher concentrations. Further analysis is needed to understand the influence of treatment, sampling dates, and temporal dynamics across different environments.

In the analysis of crop and gizzard contents, we found that difenoconazole, pendimethalin, chlorantraniliprole, fludioxonil, and azoxystrobin were the most prevalent and present at higher concentrations. A first-tier risk evaluation of these compounds, particularly those used as seed treatments, indicated a high risk of long-term effects, which should be confirmed by higher-tier (field) studies. The method will also be applied to chironomid larvae and the blood of moorhens (herbivores) and black-winged stilts (insectivores), species that breed and feed in rice fields. These samples were collected from the controlled rice fields in the study.

We present a field ecotoxicological study on pesticide use, concentrations in water, and exposure in indicator organisms. We also explore how landscape and agronomic factors influence exposure, aiming to propose a management model that balances productivity and biodiversity conservation.

2.10.P-We132 Lesser Flamingo Eggs: A ‘Lesser’ Indicator of Metal Pollution

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Flamingos are long-lived birds likely to accumulate metals from the highly saline waters they feed in. Concentrations of 24 elements in egg contents and eggshells from abandoned eggs from Lesser Flamingo *Phoeniconaias minor* breeding at Kamfers Dam, Northern Cape, South Africa, suggest metal pollution. The considerable variation in metallic element concentrations between eggs may reflect the nomadic movements of the birds over their lifetimes. Strontium concentrations in eggshells exceeded toxic reference values, and copper concentrations in egg contents may contribute towards reproductive stress. Flamingo chicks consume eggshells, which may remobilise metals such as additional Sr and Cu. Given the longevity of *P. minor* (up to 50 years), the metal concentrations were lower than expected, especially mercury. This may be due to a combination of excretion via eggs, salt glands, uropygial glands, feathers, and crop milk (among others). Selenium, known to detoxify mercury, had no association between the two element concentrations. However, the mean molar ratio was 8.2, suggesting a highly protective effect afforded by Se. Concentrations and relative compositional differences of metals show that eggshells are not a proxy for egg contents. We conclude that neither *P. minor* egg contents nor eggshells are good indicators of environmental metal pollution; feathers, blood, organs, or other species should be used. This is the first report of metallic element concentrations in egg contents of any flamingo species. Our study identified various mechanisms that may protect the embryo from metals, highlighted the contribution that eggshell and crop milk consumption may make post-hatching, and suggests the hatchling, nestling, and

fledgling stages as perhaps the more sensitive development stage than the embryo. Our findings contribute towards informing a more nuanced approach to protecting Phoenicopteridae from pollution.

2.10.P-We133 POPs Concentrations: Egg Sizes of 15 Bird Species do not Matter

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POP concentrations in bird eggs have been used for decades for monitoring and hazard assessment, published in hundreds of articles and reports. Yet, most papers considered eggs concentrations of only one or few species from a limited sample range, often with a small set of POPs classes. We investigated the effect of egg sizes (mass) of 80 eggs of 15 different species for a wide range of chlorinated, fluorinated, and brominated POPs (60+ individual compounds) collected over a large area, analysed by two different laboratories, published in two different papers. Egg masses ranged from 1.9 g for sparrows to 62 g for the African Sacred Ibis. Despite the order of differences in compound class concentrations, there was no association with egg mass, expressed as near horizontal regression slopes. The regression lines for the compound classes differed with regard to Y-intercepts but the slopes themselves did not differ between the classes. Fifteen different bird species from the same area eat different things, live in terrestrial, wetland, and aquatic habitats, have very different life histories, experience different conditions, come into contact with POPs in many different ways, differ how they metabolise POPs, and differ in how they deposit POPs into their eggs. The same goes for the chemical life histories of POPs. They have diverse sources and release pathways, differ in how they distribute between various environmental compartments even on a congener level, differ in half-lives in different compartments, and differ in bioavailability. The widely differing chemical and biological behaviours shapes the uptake and accumulation of POPs. However, as to why there was no association between independently produced and released POPs classes with bird egg mass, we have yet no answer.

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2.10.P-We134 Anticoagulant Rodenticides in Eurasian Eagle Owls: Environmental Exposure and Risk in Semi-Arid Ecosystems

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Anticoagulant rodenticides (ARs) are widely used toxic agents in rodent control, authorized for both professional and public use. Their extensive environmental presence poses a risk to non-target species, particularly birds of prey, which are vulnerable to AR contamination due to their feeding habits and high trophic level. This study assesses AR exposure in Eurasian eagle owl (*Bubo bubo*) nestlings within the Region of Murcia (southeastern Spain), an area where these nocturnal raptors exhibit high population densities across natural habitats as well as near potential AR sources, such as landfills and urban areas. This setting provides an opportunity to evaluate the influence of environmental factors on exposure risk. Blood samples were collected from 106 individuals across 34 territories in 2021 and 2022, and residues of 10 ARs were analysed using HPLC-TQ. In addition, prothrombin time (PT) was analysed (n = 62) as a biomarker of effect. Mixed linear models were employed to explore associations between selected environmental variables (human population density, land use, presence of landfills, livestock farming, proximity to watercourses) with AR concentrations and prevalence. The findings indicate a high prevalence of ARs (91.5%), with multiple ARs detected in 70.8% of samples, suggesting repeated and widespread exposure. Although detected levels were low (median Σ ARs 0.77 ng ml⁻¹), chronic low-level

exposure may pose long-term health risks. Analysis of environmental factors associated with AR contamination showed higher concentrations in more urbanized areas and near landfills, likely due to greater AR use and increased availability of target prey, such as rats. Moreover, the prevalence of two ARs (brodifacoum and difenacoum) correlated positively with proximity to watercourses, implying potential accumulation in aquatic ecosystems, particularly in semi-arid regions where water scarcity limits contaminant dilution. We observed a positive but not significant association between PT and ?ARs ($Rho = 0.04$; $p = 0.49$), suggesting that adverse effects on coagulation can be ruled out in the short term. This study emphasizes the significant prevalence and diversity of ARs detected in this species, highlighting the persistent contamination risk for raptors, which can act as sentinel species for environmental pollution. The results emphasize the need for improved regulation of ARs and for long-term monitoring of their effects on vulnerable species populations.

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2.10.P-We135 Inorganic Elements in Blood, Eggs and Embryos of Olive Ridley Turtles (*Lepidochelys olivacea*) from the Sanquianga National Natural Park, Colombian Pacific

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Lepidochelys olivacea is the most abundant species of sea turtle worldwide and in Colombia. In this country, the beaches of the Sanquianga National Natural Park (SNNP) are considered among the most important for its nesting. However, due to artisanal gold mining and cocaine hydrochloride extraction activities carried out there, it is possible that nesting females, eggs and embryos of *L. olivacea* that use these beaches are affected by exposure to inorganic elements. For this reason, in this study, eight inorganic elements (As, Cd, Cr, Cu, Hg, Pb, Se and Zn) in blood (nesting females), eggs, embryos and sand from *L. olivacea* nests in the SNNP (Colombian Pacific coast) were analysed for the first time. The samples were analysed by mass spectrophotometry with inductively coupled argon sputum, after dehydration and acid digestion by microwave. According to the results obtained, all elements were identified in all samples, and as expected, essential elements showed the highest concentrations, with Zn, Cr and Se being the most abundant elements. On the other hand, sand and embryo shells were the samples with the highest concentrations. In addition, positive correlations were identified between the elements, most of them being reported for the first time in the species. The Se:Hg molar ratio was less than 1 in most samples, indicating that not all Se is bound to Hg, due to the low concentrations of Hg present in the samples, and therefore, no negative effects on health status are expected. Likewise, there were five correlations associated with the embryo shell and none with sand, suggesting contamination by maternal transfer with higher values than those reported by other authors for the same and other sea turtle species. The results obtained provide novel information on exposure to inorganic elements in nesting sea turtles in the eastern tropical Pacific. Also reference values on the toxicological status of the *L. olivacea* population present in the SNNP are provided, which will allow future follow-up studies in this population, as an integral part of the management and conservation actions implemented in this natural area and in other areas present in the Colombian Pacific.

2.10.P-We136 Metal Contamination and Ecological Niche Variations in Sea Turtles of São Tomé and Príncipe

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The São Tomé and Príncipe archipelago hosts nesting sites for four of the seven existing and threatened sea turtle species. Due to their wide distribution, long lifespan, and diverse feeding behaviours, sea turtles often accumulate pollutants, such as metals and metalloids, throughout their complex life cycle. This study investigated metal contamination and isotopic signature in three sea turtle species *Chelonia mydas*, *Eretmochelys imbricata*, and *Lepidochelys olivacea* comparing two different life stages (nesting females, males, and juveniles) from São Tomé Island. Additionally, nesting females green (*C. mydas*) and hawksbill (*E. imbricata*) sea turtles from Príncipe Island were analysed to compare contamination levels and isotopic niches between the two islands. Female olive ridley turtles (*L. olivacea*), which nest exclusively on São Tomé Island, showed higher levels of several elements (e.g., Ca, Fe, K, Zn, Se, Cd), which could reflect natural physiological levels, contamination and/or unique dietary sources. Additionally, green and hawksbill sea turtle populations, including both adults and juveniles, showed elevated concentrations of elements such as S, Cs, and Pb. Moreover, differences in chemical elements contamination were observed between female green turtles across the two islands, despite their similar isotopic signatures. On São Tomé Island, isotopic niche analysis revealed variations in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, suggesting that green, hawksbill and olive ridley turtle populations differ in their ecological niches and/or exhibit dietary habitats, both across species and between developmental stages. This study provides insights into chemical element contamination and ecological niches across sea turtle species in the archipelago, highlighting species-specific and life-stage variations that reflect differences in diet, ecology, and environmental contamination. Furthermore, these findings enhance understanding of contamination dynamics, providing insights that can help local NGOs design targeted conservation strategies and inform policy decisions.

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2.10.P-We137 Ecological Risk Assessment of Endocrine Disrupting Chemicals Based on the Fish Physiologically-based Toxicokinetic Model

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Endocrine disrupting chemicals (EDCs) have been widely detected in various mediums due to increasing industrial and agricultural activities, which can interfere with human and animal endocrine systems and thus adversely affect human and ecosystem health. High-throughput in vitro assays combined with in vitro-in vivo extrapolation (IVIVE) leverage in vitro responses to predict the corresponding in vivo exposures and thresholds of concern. The integrated approach is also expected to offer the potential for efficient tools to provide estimates of chemical toxicity to various wildlife species instead of animal testing. However, developing wildlife physiologically based toxicokinetic (PBTK) models for IVIVE in ecological applications are challenging, especially for plausible estimation of internal effective dose (biological equivalent concentration, BEC) linked with complicated biological pathways and adverse outcomes (AO) of EDCs. Fish are most frequently used vertebrate models for ecotoxicological studies and play a key role in freshwater ecosystems. In our study, a fish PBTK model linked with IVIVE approach was established, with parameter optimization of chemical unbound fraction, pH-dependent ionization and hepatic clearance, and integration of temperature effect and growth dilution. The fish-PBTK-IVIVE approach provides not only more precise estimation of tissue-specific concentrations but also a reasonable

approximation of BEC targeting estrogenic potency of EDCs. Both predictions were compared with in vivo data and were accurate for most indissociable/dissociable chemicals. In addition, the in vivo dose-response for adverse vision and swimming disorder outcomes was also approximately characterized by BEC derived from in vitro fish assays. Furthermore, the model can help determine cross-species variability and sensitivity among various fish species. Using the available IVIVE-derived BEC with target pathways is helpful to develop predicted no-effect concentration for chemicals with similar mode of action. Due to the limited in vitro activity data available and the limited number of fish species used in the present study, results of the species sensitivity extrapolation may not be accurate enough. We conclude that the proposed fish PBTK-IVIVE approach can provide useful information for screening-level ecological risk assessment of EDCs, support the chemical environmental exposure assessment for rigorous prioritization in testing and monitoring.

2.10.P-We138 Assessing Pesticide Contamination in Freshwater Fish Along the Po River (Piedmont, Italy)

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Pesticides used in agriculture and urban areas can enter freshwater ecosystems through sewage, runoff, and rainfall. Aquatic organisms are therefore exposed to a variety of these contaminants. Fish are good bioindicators and can provide information about pesticide contamination in the ecosystem due to the different roles they play in trophic networks. In this study, fish were collected along the Po River (Piedmont, Italy) to assess the level of contamination by pesticides. Three sampling sites were selected in the metropolitan area of Turin: in the municipality of Moncalieri (260 m a.s.l.), in the centre of Turin (Murazzi) (239 m a.s.l.) and downstream of the San Mauro dam (210 m a.s.l.). All the stations have in common a certain degree of anthropogenic impact. Fish were caught by semi-quantitative sampling using electrofishing in August 2024. *Barbus* sp., *Salmo trutta*, *Telestes muticellus*, *Silurus glanis*, *Rhodeus amarus*, *Squalius* sp., *Padogobius bonelli*, *Lepomis gibbosus*, *Cobitis bilineata*, *Pseudorasbora parva*, *Leuciscus* sp., *Gobio gobio*, *Perca fluviatilis*, *Carassius carassius* were sampled, measured, and weighed, and classified according to feeding ecology and trophic level. After dissection, the dorsal part of the muscle samples (including skin) was stored at -20 °C until contaminant determination. A total of 95 pesticide compounds were screened for in muscle using gas chromatography mass spectrometry, the limit of quantification ranged from 0.002 to 0.005210 g/kg. The most common contaminants found in fish samples were bifenthrin and byphenyl, while only traces of DDT P-P' were found at all sites. The Moncalieri site showed lower contamination with no traces of endrin, endrin ketone and HCB. Traces of HCB were found only at the San Mauro and Murazzi sites in *Barbus* sp. and *Lepomis gibbosus*. The data also suggest that *Rhodeus amarus*, *Barbus* sp. and *Padogobius bonelli* accumulated more different classes of contaminants compared to the other species. These results show a disparity in contamination levels between species and contaminants, indicating possible differences in ecological or trophic exposure. The risk of ingesting contaminants through food is likely to be greater for benthivorous fish, which feed more heavily on sediment. This study highlights the importance of considering the spatial ecology and trophic role of different species when assessing pollution levels in freshwater ecosystems.

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2.10.P-We139 Assessing the Long-Term Impact of a Freshwater Oil Spill on Riverine Fish

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In 2016, over 225,000 L of crude oil entered the North Saskatchewan River (NSR) in one of the largest freshwater oil spills in Canadian history. This study aims to evaluate the long-term impact of this oil spill by determining if riverine fish are still being exposed to oil constituents and whether exposure is mediated by consumption of migratory insect prey from outside the river. The primary constituent of concern is polycyclic aromatic hydrocarbons (PAHs) which are known carcinogens with toxicity mediated by the aryl hydrocarbon receptor. Fish may feed on riverine prey exposed to the spill or a migrating species of aquatic insect called water boatmen (Corixidae). Corixidae migrate from lentic wetlands to lotic river

systems in the fall for overwinter survival and are high in omega-3 polyunsaturated fatty acids (n-3 PUFAs). N-3 PUFAs are critical components of overwinter survival in fish and thus Corixidae consumption could play a major role in offsetting PAH exposure and toxicity in fish. We caught five target species of fish using gill nets at five different sites along the NSR that range in their distance downstream from the original spill site. Fish were dissected and given internal and external health exams. PAH metabolite concentrations will be determined by analyzing bile with high performance liquid chromatography (HPLC) and synchronous fluorescence spectroscopy (SFS). Fatty acids will be determined by Gas-Chromatography/Tandem Mass Spectrometry (GC-MS/MS). Bile PAH metabolite concentrations will then be related to corixid consumption by examining the gut contents of each individual. Corixidae consumption will be further related to total lipid content and n-3 PUFA concentrations in muscle and liver. This process will be repeated over three sampling seasons in October, December, and March to track diet, lipid content and PAH exposure through the winter. Based on previous data obtained immediately following the oil spill, elevated PAH levels in fish are unexpected. However, Corixidae consumption could play a major role in the overwinter survival of fish and limit the long-term effects of the PAHs in fish exposed to contaminated sediment or prey.

2.10.P-We140 Retrospective Temporal Trend Analysis of Gene Expression Changes in Bream Livers from River Rhine (Koblenz)

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Anthropogenic substances can enter aquatic environments through emissions from urban and industrial wastewater treatment plants, leading to both short- and long-term environmental impacts. The German Environmental Specimen Bank (ESB) operates 17 sampling sites along major German rivers, collecting and storing samples, including blood, liver, and fillet from *Abramis brama* (bream) at ultra-low temperatures since the 1990s. This pilot project applied toxicogenomic methods to detect not the pollutants themselves but their biological effects by evaluating gene expression patterns in bream samples from the Rhine and comparing them to those from the relatively unpolluted Lake Belau.

Liver samples from bream collected between 1997 and 2023 at sites in Koblenz (Rhine) and Lake Belau were analyzed. Total RNA was extracted, assessed for quality, and sequenced using the Illumina NovaSeq 6000 system. Gene expression was mapped to the bream genome using StringTie and STAR. Differential expression between the Rhine and Lake Belau was calculated for each year, and overrepresentation analyses (ORA) were performed to identify biological functions affected by pollution.

RNA from archived samples had sufficient quality for sequencing (RIN > 7), and successful mapping to the bream genome revealed differentially expressed genes across years. Enriched biological processes such as organic acid metabolism, oxoacid metabolism, and sugar metabolism were identified across all years. Some processes, like fat metabolism, were enriched in specific years.

This study demonstrated the feasibility of using archived ESB samples for retrospective toxicogenomic analyses. Gene expression differences between the Rhine and Lake Belau were identified, validating the technical approach. However, attributing these differences solely to chemical pollutants is challenging, as other factors like water temperature, structure, and food supply may also influence gene expression. Overall, the study highlights the potential of long-term stored samples for environmental monitoring while acknowledging the complexities of method validation.

2.10.P-We141 Chemical Pollution & Biodiversity Loss: How to identify those chemicals that require our immediate attention?

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Biodiversity loss poses a serious threat to the health and functioning of our planet. Gaining control over this crisis is one of the most urgent tasks for mankind, as it could result in an irreversible ecological collapse and thus, destroy planet Earth as our home. It is well known that there is a link between the exposure of our environment to anthropogenic chemicals and the loss of biodiversity. However, the extent

to which chemical pollution contributes to biodiversity loss and the specific chemicals driving this loss are currently unknown. This, hence, hampers the implementation of appropriate risk management measures. Therefore, it is highly important to identify those chemicals that have the highest potential to drive biodiversity loss. Only then it will be possible to take action to reduce their emissions and the damage they cause. This poster presents a prioritization scheme that combines chemical biomonitoring data with different lines of evidence (LoEs) on respective ecotoxicity hazards and ecosystem damage. The proposed LoEs thereby bridge different levels of ecological complexity and consider various approaches on how to assess the environmental hazard of the detected chemicals. This novel approach should allow for a relatively robust identification of chemicals that might pose the largest impact on biodiversity and thus should be prioritized for follow-up monitoring and actions. Join me at my poster to learn more about the chosen approach and discuss the proposed prioritization scheme.

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2.10.P-We142 Fishing for Pollutants: Eye Lenses Open New Avenues for Reconstructing Lifetime Chemical Exposure Histories

Rebekah Boreham¹, Sara Leston², Andreia Freitas³, Philip White⁴, Boyd McKew¹, Michael Williamson⁵, Adam Piper⁵, Rosie Williams⁵, Jonathan Barber⁶ and Anna Sturrock¹, (1)University of Essex, United Kingdom, (2)University of Coimbra, Coimbra, Portugal, (3)Instituto Nacional de Investigaç o Agraria e Veterinaria, Portugal, (4)Marine and Freshwater Research Centre at the Atlantic Technological University, Galway, Ireland, Ireland, (5)Institute of Zoology, Zoological Society of London, United Kingdom, (6)Centre for Environment, Fisheries and Aquaculture Science (Cefas), United Kingdom To target pollution remediation efforts and predict the impacts of pollutants on fisheries productivity and food security, we need new tools that allow us to reconstruct lifetime exposure histories. This will allow us to understand the legacy effects of contaminant exposure on fish growth and fecundity, and the phenology, intensity and location of exposure events. However, current methods used to quantify chemical burden in fish typically use soft tissues which do not provide temporal information and can miss pulse exposures.

Eye lenses are proteinaceous structures formed of crystallin fiber cells laid down in concentric growth layers. Importantly for pollution reconstruction applications, the lens is not vascularised and the growth layers are metabolically inert creating a permanent record of the fish's life at the point of deposition. For this reason, fish eye lenses are an increasingly popular target for stable isotope analysis to track their habitat use and diet. More recently, lenses have also been used to infer mercury exposure histories, but as of yet, organic pollutants such as pharmaceuticals and PAHs have not been quantified in this tissue. Here, we developed semi-quantitative analytical methods to screen eye lens growth layers (laminae) for multiple classes of contaminants. Lens and liver samples were taken from adult demersal flatfish (dab; *Limanda limanda*) sampled all around the UK, and from European eel (*Anguilla anguilla*) sampled in rivers in northern England. Subsamples were analysed using GC-MS/MS and UHPLC-TOF-MS to compare the efficacy and contaminant types detected by the two instruments. The chemical burden observed in eye lenses was compared to that in the livers of the same individuals, which served as a benchmark for exposure assessment due to its role in metabolism and detoxification.

Successive eye laminae recorded a chronology of bioavailable contaminants from the surrounding environment, providing an exciting tool to understand patterns in pollution exposure (chronic or acute) and at what life stage(s) they were exposed. Combining lens data with location and temperature estimates derived from the otoliths (earstones) of the same animal could provide new opportunities to inform pollution mitigation and conservation efforts and to understand carryover effects of chemical exposure events.

2.10.P-We143 Heavy Metals and Microplastics in Northern Lapwing (*Vanellus vanellus*) Nesting Material: A Case Study from Lalinačka saltmarsh, Serbia

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The Northern Lapwing (*Vanellus vanellus*), a ground-nesting wader, has experienced severe population declines across Europe due to habitat degradation, agricultural intensification, and environmental pollution. Despite substantial research on anthropogenic stressors in conventional habitats, their impacts in extreme ecosystems like saltmarshes remain poorly understood. This study represents the first

ecotoxicological assessment of the relict Lalinačka saltmarsh in Serbia, focusing on the influence of heavy metals, pesticides, and microplastics on Lapwing breeding success. Fieldwork conducted during the breeding season included detailed monitoring of nesting success and the collection of environmental and biological samples - soil, vegetation, nesting material, eggshells, and feathers. The analysis identified elevated concentrations of heavy metals, including cobalt, nickel, and vanadium, in nesting material and surrounding soil, with samples exceeding established regulatory limits. Additionally, microplastic particles were detected in both the nesting material and soil, highlighting an emerging threat to avian reproductive success. Using advanced analytical techniques, including XRF, ICP-OES, and GC-MS, our work's combination of methods to analyze multiple contamination sources is both novel and interdisciplinary, providing comprehensive insights into contamination pathways. Importantly, this study fills a critical research gap as no previous studies have assessed the impacts of heavy metals and microplastics on the nesting success of *V. vanellus*. The findings illuminate the role of environmental toxins in shaping avian reproductive health in extreme environments, emphasizing the vulnerability of ground-nesting species. By integrating local case studies with global ecotoxicological concerns, this research highlights critical data for the sustainable management of sensitive ecosystems and advances the broader understanding of biodiversity conservation under intensifying anthropogenic pressures.

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2.11.P Vanishing Scales and Slimy Trails: Interdisciplinary Perspectives on Amphibian and Reptile Conservation

2.11.P-We144 Fish and Amphibian Species Sensitivity Distributions (SSDs) for Insecticides and Fungicides

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Acute fish testing is a standard data requirement for pesticide registration. The fish acute test yields a 96-h LC50, which is divided by an assessment factor of 100 in the tier 1 EU aquatic risk assessment. A common refinement option for the acute risk assessment is the species sensitivity distribution (SSD), a cumulative distribution fitted to LC50 values from at least five fish species. Then, the hazardous concentration for 5% of species (HC5) is derived from the SSD and divided in the risk assessment by an assessment factor of 9.

There is no requirement to test aquatic life stages of amphibians, but there is a requirement to assess available data (e.g., from public literature). The common approach is to assume that fish cover the sensitivity of the aquatic life stages of amphibians, which has been confirmed by several data reviews. However, it has also been suggested to increase the assessment factor further to include all retrieved amphibian data points from the public literature. Additionally, the EFSA scientific opinion on risk assessment for amphibians and reptiles urges to perform toxicity tests with aquatic amphibian life stages. However, this suggestion clearly contradicts the wish to reduce animal testing as per regulation EU 1107/2009. Finally, the EFSA Aquatic Guidance Document states that it should be checked whether a refined fish risk assessment (e.g., with an SSD) still covers the aquatic amphibian life stages.

Therefore, we fitted SSDs for fish and amphibians for 19 data-rich insecticides and fungicides, using active substance and formulation LC50 values obtained from the U.S. EPA ECOTOX Knowledgebase, supplemented with scientific and regulatory literature. Herbicides were not included due to their generally low acute toxicity and occurrence of formulation effects. SSDs were calculated for fish and amphibians separately and for the combined dataset to investigate: (1) sensitivity differences, and (2) if fish and amphibians are part of a single aquatic vertebrate SSD.

Our analyses show that for 17 out of 19 substances fish are more sensitive than amphibians, while for two substances amphibians were slightly more sensitive (within a factor of 1.5). Also, fish and amphibian data can be combined in a single aquatic vertebrate SSD. This work shows that fish are appropriate and sensitive surrogates for aquatic life stages of amphibians and as such may help in avoiding unnecessary amphibian (i.e., vertebrate animal) testing.

2.11.P-We145 Chronic Effects of Pesticide Mixtures on the Survival, Development, and Metamorphosis of the Iberian Painted Frog

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Amphibians are often excluded from pesticide risk assessments, leaving uncertainties about their protection from chemicals. Many species, such as the Iberian painted frog (*Discoglossus galganoi*), rely on rapid development to leave temporary ponds, making them particularly vulnerable to environmental disruptions. In this study, we investigated the chronic effects of the herbicide 2,4-D and the insecticide flupyradifurone on *D. galganoi*. To do so, egg clutches of this species were collected from the wild and reared in the laboratory before being assigned to 12 treatment groups, including five 2,4-D concentrations (0.03 100 ppm), two flupyradifurone concentrations (10 or 100 ppm), four combinations of the two pesticides, and a control group. Tadpoles were exposed from early development stages to metamorphic climax, with survival and growth closely monitored. Metamorphosing animals were individually housed until tail resorption for final measurements and pesticide residue analyses. High concentrations of 2,4-D caused severe mortality during aquatic stages, with complete mortality at 100 ppm within the first day of exposure. Combined treatments with flupyradifurone resulted in faster mortality compared to single-substance exposures, indicating additive effects. Average body condition during aquatic development was significantly higher in the control group than in the 30 ppm 2,4-D and combined treatment groups. Animals treated with 0.3 ppm of 2,4-D alone showed slightly better outcomes than those in flupyradifurone combinations, though high variability likely masked statistical significance. Development times were significantly extended for some of the treatments, particularly at 30 ppm of 2,4-D, delaying metamorphosis climax by more than 15 days compared to controls. Among individuals reaching metamorphosis climax, all treatment groups except the control caused mortality before completing metamorphosis, and malformations were prevalent, with the highest rates observed in the 3 ppm 2,4-D group and frequently occurring in combined treatments. These findings underscore the heightened vulnerability of amphibians to pesticides during critical developmental stages and highlight the need for comprehensive risk assessments and research that address their unique ecological challenges.

2.11.P-We146 Assessment of Methylmercury and its Independent and Synergistic Effects on Adult Survival Across Multiple Populations of Amphibians

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Amphibians face unprecedented global declines from many threats, including contaminants such as mercury and their interaction with other stressors. While the biphasic life history of many amphibians increases the risk of methylmercury (MeHg) exposure in aquatic habitats, the broad-scale distribution of MeHg exposure in amphibians and its effects on animal health remains largely unknown, whether considering MeHg alone or in combination with other stressors like disease. We used nonlethal sampling to assess MeHg bioaccumulation in juvenile and adult amphibians from 26 populations (14 species) in the USA during 2017-2021, including several imperiled species that could not have been sampled by traditional lethal methods. For 10 of these same populations (7 species), we conducted multi-year capture-mark-recapture studies and used hierarchical models to estimate how MeHg affected survival of individual amphibians directly and in combination with *Batrachochytrium dendrobatidis* (Bd), the fungus that causes the lethal disease amphibian chytridiomycosis. We found a 33-fold difference in tissue concentrations of MeHg from amphibians across study sites. Much of the variation in bioaccumulation was linked with life history characteristics of sampled species such as size and trophic position. Our capture-mark-recapture study revealed MeHg can directly reduce survival of individuals and act synergistically with Bd to further reduce survival. Although effects varied among species and sites, our results provide the first evidence of mercury (Hg or MeHg) reducing survival of wild, adult amphibians. Our results also help clarify the potential for direct and synergistic effects of MeHg and disease and emphasize the complexity of identifying and quantifying interactions among stressors under field conditions.

2.11.P-We147 A Dynamic Model to Assess the Combined Effects of Batrachochytrium Dendrobatidis Infection and Pesticides in Amphibians

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The global amphibian decline is likely multi-causal. Among other contributing factors, combined effects of chemical toxicity and infectious diseases are likely contributing to population and biodiversity decline. Dynamic models can help to improve mechanistic understanding of such multi-stressor effects and assess possible interactions in a wide range of scenarios.

To model the combined effects of pesticides and *Batrachochytrium dendrobatidis* (Bd) infection on the life-history of amphibians, we developed a dynamic model consisting of three components: A model of the life-history of amphibians, based on Dynamic Energy Budget (DEB) theory, a model of Bd growth, infection and effects, and a Toxicokinetic-Toxicodynamic (TKTD) model to account for effects of pesticides. The Bd life cycle was modelled based on a previously developed model which accounts for density-dependent growth of sporangia, and was extended to account for variability in Bd infection susceptibility among amphibian individuals.

We estimated DEB parameters for the anuran *Discoglossus galganoi* from larval and metamorph growth and developmental data.

Parameters accounting for Bd growth and infection dynamics were estimated from the distribution of measured Bd loads across *D. galganoi* individuals. The parameters describing effects of Bd loads were inferred from effects on the 10-day growth rate of juveniles, commencing 10 days after exposure to Bd. All parameter estimations were done using a likelihood-free Bayesian approach. The full set of parameters was not identifiable from the data, highlighting the need for approaches which account for parameter uncertainty (e.g., Bayesian inference) and reduce uncertainty (e.g., pooling data from multiple sources). Overall, the developed model implementation is highly flexible and allows to extrapolate effects across a wide range of scenarios. Emerging challenges in the calibration of this model lie in the number of parameters, as well as the stochastic nature of Bd infection dynamics. The presented model can help to illuminate possible interactions between Bd infection and chemicals, e.g., by assessing the effect of a chemical's mode of action on the probability of an interactive effect between the chemical and Bd infection on apical endpoints. In combination with further experimental data on the effects of chemicals, this can in turn support the risk assessment of pesticides for amphibians.

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2.11.P-We148 Can Abiotic Factors Related to Climate Change Affect Larval Stages of *Pelophylax perezi*?

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Climate change is a growing concern for the scientific community. It is predicted that climate change-driven alterations will be more profound for the Iberian Peninsula, namely in high-altitude regions. The predictable alterations will have profound implications on biota, with amphibians being suggested as one of the most vulnerable classes due to their unique characteristics and high susceptibility to environmental factors. Additionally, the sensitivity to environmental alterations may vary depending on the background of the populations. This background information is relevant not only to predict the effects of environmental alteration in different regions but also for those conducting ecotoxicological studies resorting to animals from populations with different backgrounds. With this in mind, our study aimed to assess the effects of climate change related abiotic factors (pH and temperature) in amphibian larvae with two distinct altitude background (below 50 m and above 900 m). To achieve this, a full factorial experimental design was performed, with 3 different pH levels (5.5, 6.5 and 7.5) and 3 different temperatures (16, 20, 24 °C) using larvae from both altitudes. Furthermore, to assess the effect of water volume reduction a second assay was carried out, consisting in assessing the effect of several exposure volumes/water column heights in tadpoles from high altitude which will be more prone to be affected to desiccation effect. The endpoints measured for both approaches were both morphological (size) and physiological (activity of antioxidant defence system enzymes and peroxidative damage). From our approach the main results showed that temperature has higher influence than pH in the development time and also in the size in tadpoles, being the effects dependent on the origin (low or high altitude) of the animals. Also, the physiological parameters suggest that animals from high-altitude present higher stress to high temperatures. Furthermore, the scenario of the lowest volume/water column height (desiccation) also revealed high animal stress. Overall, the most relevant results of the present study, indicate that the alteration in abiotic factors, mainly temperature has influence on the development of *P. perezi* and that high-altitude populations may be more severely affected in a scenario of climate change where temperature increases, and desiccation occurs.

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2.11.P-We149 From Indicators to Allies: Lizards' Ecological Role and Toxicological Insight in Agriculture Environments

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The application of agrochemicals has become an important tool to maximize harvesting, although their intense use can pose direct and indirect ecotoxicological risks to non-target organisms, such as reptiles. Lacertid lizards are pervasive in agricultural landscapes where they play a key functional role, yet the extent of their exposure to pesticides and their mechanisms for coping with these chemicals remain poorly understood. This study aims to determine whether and how agricultural practices can influence and interfere with lizard fitness (lizards health status) and their trophic networks (diet composition), ultimately affecting the quality of their ecosystem services. Lizards were sampled from seven vineyards sites undergoing different pesticides applications in northern Portugal. Morphometric traits, blood smears (parasite quantification), fecal pellets and tail tips for diet analysis (metabarcoding and isotopes) were collected. Moreover, microhabitat quality was assessed along transects through data loggers, thermos-pictures and pictures to record habitat structure and vegetation. The results revealed slightly variations among pesticides applications in body condition, parasitisation and in dietary patterns. Although some associations remain still unclear due to the complexity of natural systems, yet the observed trends provide valuable insights and warrant further investigation for the conservation of reptiles in agricultural environments. Furthermore, the outcomes of this study are expected to contribute significantly to both functional ecology and agricultural management by providing quantitative evidence to inform decision-making. An integrated functional ecology and ecosystem services framework may be an effective approach to advancing sustainable agriculture and improving conservation practices.

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2.11.P-We150 A Comprehensive Dataset of Amphibian Physiological and Biochemical Parameters for Toxicokinetic Modeling and Comparative Analysis

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Amphibians are one of the most diverse vertebrate groups on Earth, playing an important role in terrestrial and freshwater ecosystems. However, they are experiencing the fastest rate of decline among all vertebrates, with populations facing multiple threats worldwide. To navigate their protection from environmental chemicals, read-across from other classes (e.g., mammals) is used. This however could potentially be improved by habitat specific toxicokinetic modeling for which a deeper understanding of their physiology is essential. This study addresses this need by presenting a comprehensive dataset on various relevant physiological and biochemical parameters.,

We conducted a literature review using broad queries combining "amphibia" with specific traits related to physiology (e.g., "skin permeability", "organ weight", "blood plasma"). Over 700 abstracts were retrieved from Web of Science; for 150 entries the full text was retrieved to extract the measurements. Data sources included information extracted from tables and graphs, calculations derived from supplementary information, and details obtained through direct contact with the authors. We differentiated data by species, sex, age, in situ / ex situ, along with trait-specific distinctions.

Preliminary work identified, data for organ weights, expressed as a percentage of body weight, for 27 amphibian species. Lipid content for the whole body and individual organs has been gathered for 30 species across twelve different families. The dataset also includes detailed tissue specific and whole-body composition data, such as moisture, lipid (composition), protein, lean mass, and glycogen percentages. These parameters were best reported for *Lithobates catesbeianus*, which is farmed on an industrial scale, and for the model organism *Xenopus laevis*, but data for various species within the family Hylidae and Salamandridae were also retrieved. For the liver, which plays a key role in the degradation of chemicals, different characteristics were documented for 38 species.

Ongoing work aims to expand the dataset. This dataset supports the comparative analysis of species-specific biochemical profiles, providing insights into physiological variation within and across taxonomic groups, sexes, habitat and study conditions. Additionally, it is the basis for future physically based toxicokinetic modelling for amphibians which could improve their risk assessment, leading to better protection.

2.11.P-We151 From Soil to Skin: Predicting Pesticide Uptake in Caudates

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Dermal uptake is considered one of the key drivers for amphibian exposure to pesticides. Indeed, multiple

studies have confirmed the relevance of this uptake pathway, which is generally considered to be the result of their highly permeable skin. We recently, described a modelling approach that aims at deriving body burdens resulting from exposure via contaminated soil. However, these efforts have been mainly focused on anurans, as exposure data in caudates is scarce.

Here we present an updated one-compartment Toxicokinetic (TK) model that aims at deriving realistic body burdens resulting from exposure via contaminated soil in caudates. The TK model was adapted to describe uptake mechanisms (passive, active, rehydration) in caudata, and was validated for 5 pesticides. Parameters used in the model included tested species, body weight, soil concentration, body burdens, skin surface area, skin thickness, K_{oc}, permeability coefficient of the pesticide and water uptake rate of the animals. Poly-parameter Linear Free Energy Relationships modelling was used to describe the distribution of the corresponding pesticide between water and amphibian tissue and integrated into the equation. Modelled body burdens were subsequently compared to actual, measured body burdens for a total of 25 individuals exposed to 5 different pesticides. In order to compare the overall predictability of the model for a given pesticide, predictions and measured concentrations were averaged over all individuals for a given pesticide. Overall, a good concordance between modelled and measured body burdens was observed, comparable with the previously described anuran model. Results were subsequently plotted on a log-log plot and a regression analysis performed, resulting in an R² of 0.62 and a correlation coefficient of 0.67, suggesting adequate predictivity of the model.

Based on these results, it is possible to derive realistic body burdens using TK modelling in caudates, although more data would be desirable to confirm applicability to a wider range of pesticides. We suggest that similar to anurans, TK models could be used to characterize dermal exposure in caudates, to screen for pesticides of concern, reducing the overall need for animal testing.

2.11.P-We152 From Fish to Frogs: Leveraging Fish Toxicity Data to Predict Chronic Pesticide Effects on Aquatic Amphibian Life Stages

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Existing data indicate that fish toxicity is predictive for pesticide toxicity on aquatic life stages of amphibians. While many studies have confirmed this for acute toxicity, predicting chronic toxicity is more challenging. Since 2018, the European Food Safety Authority has mandated the submission of amphibian studies for pesticides as part of its Guidance for identifying endocrine disruptors. This requirement has significantly increased the availability of Amphibian Metamorphosis Assay (AMA) data, allowing for an update of former correlations between fish Early Life Stage (ELS) and AMA data. This project aims to evaluate the relative sensitivity of amphibians compared to fish using existing AMA-ELS data. We extracted data on apical endpoints survival, body weight, and length from ELS and AMA study reports used for plant protection product registrations, as well as publicly available sources. In our analysis, we focused on No Observed Effect Concentrations (NOECs), which are routinely used in environmental risk assessments (ERA). We assessed the statistical correlation between fish and amphibian NOEC values using Spearman's rank correlation. Ultimately, we aim to develop an interspecies correlation estimation (ICE) model to predict chronic aquatic amphibian sensitivity based on fish ELS endpoints. Preliminary data for 65 pesticides show that aquatic-phase amphibians and fish exhibit similar chronic sensitivities to pesticides, showing a significant correlation. Fish were found to be more sensitive to a greater number of pesticides. For only <10% of assessed pesticides frogs were 10-fold more sensitive than fish regarding body weight and length. Considering survival, a 100-fold higher sensitivity was found for a single pesticide only. It is important to note that the limited number of test concentrations in the AMA may result in low NOECs, which introduces some bias into the analysis. Furthermore, the endpoints derived from the AMA are specifically designed for endocrine screening and are not intended for use in ERA. Future work will focus on expanding the database, refining endpoint calibration, evaluating the impact of unbound NOECs on the analysis, and examining variations between pesticide classes. The ultimate goal is to enhance amphibian protection in the ERA of plant protection products while minimizing the need for additional vertebrate studies. To this end, we will derive an ICE model to predict amphibian sensitivity based on ELS endpoints.

2.11.P-We153 Evaluating the Direct and Indirect Effects of Chemical Contamination on Amphibians in the Interface Between Water and Land in Agricultural Landscapes

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Chemical contamination is widely recognised as one of the main drivers of biodiversity loss, which is especially concerning for amphibians, currently the most threatened vertebrate group. Due to their unique physiological and biological characteristics, amphibians are particularly vulnerable to the threat posed by

chemical contaminants. A case of especial concern pertains to pesticide use and application on agricultural land, which can severely impact the species living in or around cultivated fields. Although there are regulations in place to protect the environment from such threats, these regulations are mainly based on Environmental Risk Assessments (ERAs) that are both limited and fragmented. Furthermore, amphibians are often neglected in the ERA and subsequently not adequately protected. The current framework contemplates separated ERAs for aquatic and terrestrial environments, as well as for different chemicals. However, this framework does not represent the real-life scenario of amphibian exposure to pesticides in agricultural fields, as it overlooks the possibility of multiple exposure routes and the potential of occupancy of both aquatic and terrestrial compartments during the course of their life cycles and foraging periods. As such, the present work aims to understand the flow of pesticides from agricultural fields to the nearby ponds in central Spain; record their risks for the amphibian populations developing in these ponds; and study how the effects on these populations mediate the return of contaminants, along with their nefarious consequences, to the terrestrial habitat. The ultimate goal is to generate data to provide a reference scenario for an ERA scheme combining aquatic and terrestrial environments in a more holistic approach. To do so, the present study proposes to focus on assessing food availability, diet composition, susceptibility to diseases, body condition, species richness and diversity, demographic structure and reproductive output of field populations of amphibians whose aquatic development is completed in ponds generated by the runoff water of the neighbouring cultivated fields, paralleled with the experimental evaluation of long-term effects of early aquatic exposure manifested in terrestrial adults. A gradient of detrimental effects positively associated with the degree of contamination is expected to be observed.

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2.11.P-We154 The Impact of Agricultural Contaminants on Populations of the European Common Toad

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Amphibians are the most threatened class of vertebrates worldwide, according to the IUCN Red List. Habitat loss and degradation are considered to be major threats causing population declines. Even common and widespread species, such as the European Common toad (*Bufo bufo*), are facing gradual population declines. Even a minor decline of a common species results in a significant decrease in biomass, which is expected to have major effects on ecosystem functions and services. Therefore, it is crucial to understand the causes of toad population decline in order to formulate effective conservation strategies.

Agricultural contaminants were analysed in water samples from ten ponds with declining toad populations and compared to ten ponds with stable populations, selected throughout different ecoregions in Flanders (Belgium) and sampled monthly over a period of 3 months. Pesticides ($n = 112$), mycotoxins ($n = 20$), and antiparasitic drugs ($n = 15$) were analyzed by targeted ultra-high performance liquid chromatography-tandem mass spectrometry methods (UHPLC-MS/MS). Furthermore, 46 antimicrobial drug residues were detected using UHPLC combined with high resolution MS (UHPLC-Orbitrap-HRMS). For the analysis of 11 heavy metals, both inductively coupled plasma - optical emission spectrometry (ICP-OES) and ICP-MS were used. In addition, nutrient ($n = 5$) levels were measured using ion-exchange chromatography (IC), and total nitrogen content was determined using a Kjeldahl digestion combined with a distillation-titration method.

An exploratory data analysis of a subset of samples indicates that declining populations are linked to slightly higher total concentrations of nutrients and pesticides, but heavy metals and total nitrogen content do not appear to be related to population status. Other analyses are currently in process. A Principal Component Analysis (PCA) will be carried out to explore the relationships between the measured concentrations of contaminants and population status, and potential interactions between contaminants. By linking toad population decline to the presence of certain contaminants, we can provide effective conservation strategies to mitigate threats to an ecologically valuable species. Future research will focus on contaminants that were identified as possible drivers of population decline, in order to fully understand how these contaminants affect toad health.

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2.11.P-We155 Interaction Between *Pelophylax perezi* and the Amphibian Pathogen *Saprolegnia australis* under Salinisation Scenarios - What Doesn't Kill You Makes You Stronger?

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Oomycetes from the genus *Saprolegnia* constitute one of the groups of pathogens contributing to the global amphibian decline. Considering that amphibians increasingly live in areas altered by anthropogenic activities, the interplay between host-pathogens-environment has emerged as a critical aspect for understanding the spread and severity of saprolegniosis under environmental perturbations. This study intends to contribute for a better understanding of the effects of rising salinity levels - driven by climate change-sea level rise, and subsequent seawater intrusion in coastal areas - on the interaction amphibian-*Saprolegnia*. It is hypothesised that tadpoles sensitivity to salinisation is influenced by the pathogen *Saprolegnia*. For this, a full-cross factor experimental design was performed by exposing tadpoles of the Perez's frog *Pelophylax perezi* to two salinity levels (obtained by dilution of natural seawater) in the presence or absence of the pathogen *Saprolegnia australis* and to a control (without seawater and *S. australis*), for 14 days. Tadpoles were exposed to *S. australis* using two methodologies: (1) full contact, where seeds inoculated with mycelium were deposited on the bottom of the test vial; (2) and partial contact, where seeds inoculated with mycelium were introduced inside a net of 50 µm pore to allow exposure only to the spores. In both cases, a protocol of sporulation induction was performed. Mortality, weight, growth, developmental stage and macroscopic signs of infections were the endpoints observed during the experiment. The obtained results revealed that the presence of *S. australis* influenced the sensitivity of tadpoles to salinity. Overall, the presence of *S. australis* in full contact with tadpoles caused more mortality compared to the salinity treatments in the absence of the pathogen or when tadpoles were in partial contact with it (only for the lowest salinity level). Furthermore, *S. australis* in full contact with tadpoles induced their faster development and an increase in their biomass and growth. This hypothesis was further tested with tadpoles originated from natural populations with different historical background of exposure to anthropogenic stressors to identify if this pattern of responses persists.

2.11.P-We156 Different Faces, Same Dangers? Comparative Hazard Assessment Of Bisphenol A And Its Analogue Bisphenol S-MAE With In Vivo And In Vitro Amphibian Models

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With the growing demand for plastic products, the environmental release of plasticizers is expected to increase. Bisphenol A (BPA), one of the highest-volume industrial chemicals, is widely detected as a contaminant in aquatic ecosystems worldwide. Due to its estrogenic, neurotoxic, and genotoxic properties, as well as its potential for bioaccumulation and biomagnification, BPA is classified as a substance of very high concern. In response to its hazards to human and environmental health, the European Union has prioritized finding safer alternatives. Bisphenol analogues, such as bisphenol S-MAE (BPS-MAE), have been proposed as possible replacements; however, their similar physicochemical properties suggest they may also pose similar toxicity hazards. Due to their recent production and usage, limited information is available on organisms susceptibility to these xenobiotics, particularly for vulnerable groups like amphibians. This study aimed to make a comparative assessment of the toxicity of BPA and BPS-MAE using *Xenopus laevis* in vivo and in vitro models. It also aimed to evaluate the suitability of in vitro assays as non-animal alternatives for first-tier risk assessment of amphibian aquatic stages. Tadpoles were exposed to various concentrations of BPA and BPS-MAE for 96-h, while A6 and XTC-2 cell lines were exposed to equivalent concentrations for 72-h. This work provides the first evidence of BPS-MAE toxicity on amphibians. In tadpoles, BPA had a significant effect on survival, malformations and body growth, with 96-h LC50, EC50, and LOEC values of 11.4, 10.0, and 5.3 mg/L, respectively. BPS-MAE affected these endpoints but also heartbeat, at slightly higher values of 18.1, 18.0, and 15.5 mg/L. Considering cytotoxicity, for A6 cells the 72-h LC50 values of BPA and BPS-MAE were 18.2 and 21.7 mg/L, respectively, while for XTC-2 cells were 26.4 mg/L for BPA and 22.9 mg/L for BPS-MAE. Overall, XTC-2 cells were more resistant than A6 cells to both bisphenols, and tadpoles were more sensitive than either cell line. The overlapping confidence intervals of the computed toxicity parameters between in vivo and in vitro models support the viability of in vitro assays as reliable alternatives for initial risk assessments in amphibians. Additionally, although BPA demonstrated higher toxicity, the similarity of LC50, EC50, and LOEC values between BPA and BPS-MAE highlights the unsuitability of the latter BPS-MAE as a safe environmental alternative to the former.

2.11.P-We157 Toxicity and Behavioral Effects of Wildfire Ash on *Xenopus laevis*: The Role of Portuguese Native and Exotic Vegetation

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Recent wildfire seasons in Europe have been increasingly destructive, severely impacting ecosystems and biodiversity. In Portugal, wildfires devastated forests, including of both native and exotic species. These wildfires introduce ash with toxic compounds, such as polycyclic aromatic hydrocarbons and metals, into aquatic ecosystems. Vegetation cover can affect ash composition and subsequently its toxicity to aquatic organisms, yet its specific effects on amphibians, which are important indicators of the ecosystem's health, remain underexplored. This study assessed the influence of wildfire ash elutriates, originated from Portuguese native *Arbutus unedo* (strawberry tree) and from exotic *Eucalyptus globulus*, on the behaviour and other fitness related parameters of *Xenopus laevis* tadpoles. To achieve this goal, two experimental setups were performed: (1) a 96-hour acute toxicity assay was performed by exposing tadpoles to a range of concentrations of aqueous ash extracts (AAE) from each vegetation cover (0.625–10.0 g L⁻¹). At the end of the assay the following endpoints were measured: mortality, body lengths (tail, snout-to-vent, and interorbital), and body weight. (2) a 12-h avoidance assay in the HeMHAs system, where tadpoles of *X. laevis*, previously fed or non-fed, were exposed to a gradient (0.625–10.0 g L⁻¹) of AAE from both vegetation covers. At the end of this assay, the avoidance from AAE contaminated compartments was quantified.

In the 96-hour assay, no significant mortality was observed, but significant decreases in body length and weight (LOEC = 6.25 g L⁻¹) occurred across all concentrations, with the most severe effects at the highest concentrations of *E. globulus* AAE. In the avoidance assay, neither *E. globulus* nor *A. unedo* affected the relative distribution of *X. laevis* tadpoles across the compartments of the HeMHAs system. However, both AAE led to avoidance behaviour in previously fed tadpoles, with *E. globulus* (LOEC = 6.25 g L⁻¹) inducing a greater avoidance response than *A. unedo* (LOEC = 12.5 g L⁻¹). These results suggest that *Eucalyptus* ashes have a stronger adverse effect on tadpoles compared to *A. unedo* AAE.

These findings indicate that the vegetation type burned significantly influences ash toxicity. The avoidance and toxicity assays highlight *E. globulus* as more harmful to *X. laevis* tadpoles, potentially affecting their growth and survival. Further research is needed to understand how these impacts might affect their development.

2.12 Ecotoxicology and Environmental Chemistry in Mountain and Remote Areas: Challenges, Impacts, and Future Directions

2.12.T-01 Polycyclic Aromatic Hydrocarbon (PAHs) Contents in Soil Organic Matter Fractions Along an Elevation Gradient in the French Alps

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Polycyclic aromatic hydrocarbons (PAHs) are toxic, ubiquitous and persistent organic pollutants in the environment, mainly produced by road traffic and domestic heating. These pollutants are semi-volatile compounds which, once released into the atmosphere, can be transported over long distances and reach remote areas such as mountainous regions.

PAHs have a high affinity for soil organic matter (SOM) and therefore tend to accumulate in soils after deposition. Thus, PAH and SOM dynamics cannot be dissociated. We used a common approach of SOM studies (density fractionation) to better understand the PAH dynamic in mountain soils, along an elevation gradient (Lautaret, France). This approach consisted to study 3 different SOM fractions: the free particulate organic matter (fPOM), the occluded particulate organic matter (oPOM) and the mineral-associated organic matter (MaOM) and to measure PAH and organic carbon (OC) contents in bulk soils and SOM fractions. SOM quality were analysed by nuclear magnetic resonance (NMR) spectroscopy for fPOM and oPOM fractions, and by attenuated total reflectance (ATR) for MaOM fraction.

The results showed that total PAH contents in bulk soils ranged from 55 to 239 ng·g⁻¹, corresponding to typical regional background values for PAH contamination in mountainous areas. In the fractions, the highest PAH contents were found in the oPOM fraction, followed by the fPOM fraction then the MaOM fraction. The results revealed that the distribution of PAHs in the soil is partly determined by the amount of OC in each fraction. The NMR results showed that PAH contents were strongly correlated with the

increase degree of decomposition of SOM. This suggests that the distribution of PAHs may be linked to the transformation of fractions (from fPOM to oPOM), also associated with the formation of aggregates (providing physical protection for PAHs). With regard to the MaOM fraction, the lower PAH contents can be simply explained by the nature of this fraction, which consists of microbial- and plant-derived SOM. The results of this research indicated a preferential distribution of PAHs within soils (oPOM > fPOM > MaOM), driven by the OC contents and probably link to the formation of aggregates in soils. As a such distribution may have an impact on the dynamic of pollutants in soils, it can be taken into consideration in future research.

2.12.T-02 Airborne Benzothiazoles and Organophosphate Flame Retardants at a High-altitude Background Site in the Eastern Italian Alps: Occurrence, Sources and Temporal Trends

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In the last decades, rapid urbanization and industrial and economic growth have led to the acceleration of the anthropogenic impact on the planet and even remote areas are no longer pristine. The emission in the environment of Chemicals of Emerging Concern (CECs) is consistently increasing, hence their monitoring is of growing interest for the scientific community and policy makers. Among other environmental compartments, the atmosphere is one of the most affected by the growing number of CECs, as they are still poorly regulated and not extensively monitored.

Both benzothiazoles (BTHs) and organophosphate flame retardants (OPFRs) are listed as high volume production chemicals due to their extensive use in a wide range of industrial and consumer products, and their increasing release in the environment poses risks for the ecosystems and human health, as different studies have demonstrated their potential toxic effects.

This study aimed to investigate the occurrence of eight BTHs and six OPFRs in PM₁₀ samples collected at a high-altitude background site located in the Eastern Italian Alps, to observe temporal trends and to assess their sources and transport processes.

Aerosol sampling was carried out from April to October 2023 at Col Margherita Observatory (2543 m a.s.l.) using a PM₁₀ low volume sampler. Analytical determination was performed via UHPLC-MS/MS. NOAA ARL s HYSPLIT model was used to determine the origin of air masses reaching the study area. The effect of typical alpine phenomena, such as the valley breeze, on atmospheric transport of the target compounds was evaluated by calculating the daily average temperature lapse rate considering the main valleys that influence the air fluxes to the study site.

Results confirmed the presence of both BTHs and OPFRs in alpine aerosol, with concentrations ranging from pg to ng m⁻³. Both classes of contaminants exhibit a seasonal trend, with higher concentrations in spring, probably due to a combination of atmospheric processes such as thermal convection, mountain-valley breeze regimes and upward motion due to mixed-layer expansion.

The present study provides for the first time information on background concentration levels of BTHs and OPFRs in the atmosphere of an high mountain remote area, offering insights into their sources and transport mechanisms that will help for future investigations on the pathways and environmental fate of these classes of chemicals.

2.12.T-03 High-Mountain Lakes as Indicators of Microplastic Pollution: Insights From the Alps

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Microplastics can reach remote areas, such as alpine lakes, through atmospheric transport and deposition. These ecosystems are sentinel systems that can provide information on the effects of climate change and anthropogenic pressures. This study aimed to investigate the presence of microplastics (MPs) in abiotic matrices (water and sediments) and aquatic organisms (zooplankton, tadpoles (*Rana temporaria*), fish (*Salvelinus fontinalis*)) in two high mountain lakes (Upper Lake Balma and Lower Lake Balma) in the Cottian Alps (northwest Italy). Biotic and abiotic samples were processed in the laboratory and MPs were detected by Fourier transform infrared spectroscopy.

The MPs found in the biotic samples were compatible in shape (fibers and fragments), colour (black, blue, white) and chemical type (polypropylene and polyethylene) with those found in the abiotic samples. No MPs were detected in the water samples. The average MPs in the sediment samples were 1.33 ± 0.67 items/m³ and 1.75 ± 0.62 items/m³ in the Lower and Upper Lake Balma, respectively. No MPs were

found in zooplankton: this could be due to the absence of MPs in the water and the ability of copepods to shed microplastics while foraging. The mean number of MPs in tadpoles was 0.33 ± 0.58 items/individual and 0.66 ± 0.58 items/individual in Lower and Upper Lake Balma, respectively. Further studies are needed to investigate the effects of MPs on tadpole development and growth. The mean number of MPs elements found in the gastrointestinal tract (GIT) of fish was significantly higher in samples from the Lower Lake (0.45 elements/g GIT) than in those from the Upper Lake (0.20 elements/g GIT). A negative relationship was found between fish size and MPs abundance in GIT, indicating that juvenile fish accumulate more MPs, probably due to the high prey ingestion rate compared to adults. This study represents a comprehensive assessment of the presence of MPs in the biotic and abiotic compartments of a high mountain lake. Although the number of items in the samples was low, our findings suggest long-range transport contamination. *Salvelinus fontinalis* could be considered as a good bioindicator of MPs pollution in alpine lakes, and as most of these environments were originally fishless, fish could be removed for MPs monitoring in high mountain environments. Further studies are desirable to better understand the source and dynamics of MPs in high mountain lakes.

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2.12.T-04 Using Dragonflies as Bioindicators of Per- and Polyfluoroalkyl Substances (PFAS) Exposure in United States Protected Areas

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Per- and polyfluoroalkyl substances (PFAS) are ubiquitous in the environment and are of significant global concern to human health, wildlife, and ecosystems because of their prevalence, persistence, and toxicity. Because of their wide variety of uses, PFAS are found in many environments and organisms, including in atmospheric particulate matter and wet deposition. Organizations worldwide have emphasized a critical need for understanding the occurrence, fate, transport, and effects of PFAS in the environment and to the public. Currently, there is an incomplete understanding of PFAS exposure within ecosystems and food webs, especially on protected lands including remote and mountainous areas, limiting our understanding of whether sensitive natural resources are at-risk. Dragonfly larvae are considered an ideal biosentinel for bioaccumulative contaminants due to their predatory nature, prevalence in a variety of water bodies, and widespread geographic distribution across six continents. They are also easy to sample making them an ideal species for large network designs including those incorporating aspects of participatory science. The current study was designed to address the prevalence of PFAS on protected lands, determine the ecologically relevant PFAS mixtures occurring in biota, and quantify the utility of dragonfly larvae as biosentinels for PFAS to address potential risks to fish and humans. To accomplish these objectives, we utilized an existing citizen-science network to collect dragonfly larvae from over 90 national parks and wildlife refuges across the United States (US) from 2021-2023. After collection, samples were dried, composited (N = 160 composites) and analyzed for 28 individual PFAS. Based on preliminary data from about half the samples, at least one PFAS was observed in each composite dragonfly larvae collected and PFBA, PFOS and PFTrDA were observed most frequently at concentrations ranging from <1-480 ng/g dry weight (median: 2.5 ng/g dry weight). Results from this initial broad scale assessment of PFAS on protected lands will provide parks and refuges with information needed to proactively manage their resources, particularly in areas where PFAS contamination is an issue. Information from this study will also identify localized contamination hotspots, prioritize future monitoring and modeling efforts, and establish future management strategies with the goal of minimizing or reducing PFAS contamination across US protected areas.

2.12.T-05 No Longer Pristine! Occurrence of Perfluoroalkyl Chemicals (PFAS) and an Herbicide in Water Bodies in the Tropical Rainforest of Madagascar

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The increasing presence of chemical pollutants in even the most remote regions raises concerns for both human health and ecosystem integrity. This is especially important in biodiversity hotspot areas, where pollutants can disturb threatened species and habitats. This study aimed to assess the occurrence of chemical contaminants, including perfluoroalkyl substances (PFAS), herbicides, biocides, and other micropollutants, in the tropical mountain rainforests of Eastern Madagascar an area traditionally

considered to be minimally affected by chemical pollution. Water samples were collected from five sites with varying levels of anthropogenic disturbance and forest fragmentation: Maromizaha (MZ), Mitsinjo (MJ), Mahatsara (MH), Andasibe (AD), and Anevoka (AK). We performed a target analysis on 747 micropollutants that are known to be widespread globally and a separate analysis on 13 PFAS compounds. Our findings revealed the presence of eight PFAS compounds, with five (PFPeA, PFHxA, PFHpA, PFOA, and PFDA) detected in more than half of the samples, at concentrations ranging from 0.56 ng/L to 2.13 ng/L. We also detected several other pollutants, including the herbicide Dinoterb, which exceeded its Predicted No Effect Concentration (PNEC) in some sites, particularly in the village of Andasibe, where agricultural activities are constantly carried out. It is worth noting that we found low concentrations of different chemical pollutants even inside the protected area of Maromizaha, which was supposed to be pristine. Overall, while most pollutants were found at levels below their respective PNEC values, these results underscore the growing threat of chemical pollution in tropical areas that already facing habitat fragmentation and climate change. Chemical pollution in the area suggests potential underestimated risks to both human health and the biodiversity of Eastern Madagascar's rainforests. These findings call for continued monitoring and protective measures to safeguard these vulnerable ecosystems.

2.12.P Ecotoxicology and Environmental Chemistry in Mountain and Remote Areas: Challenges, Impacts, and Future Directions

2.12.P-We158 Understanding Drivers of PFAS Contamination in Seabirds: Insights from Biologging Devices

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Per- and poly-fluoroalkyl substances (PFAS) are synthetic, persistent and ubiquitous compounds. Although PFAS occur widely in wildlife tissues, few studies have investigated the link between PFAS exposure and habitat use during reproduction and migration. Deployment of tracking devices (bio-logging) could provide invaluable insights into the identification of spatial origins of contamination. Seabirds are commonly used as bioindicators of marine pollution, especially gull species (*Larus* sp.) which use a variety of coastal, pelagic, urban and terrestrial habitats, potentially providing crucial information on the geography of PFAS contamination. Here, we combined blood analyses of 11 PFAS and stable isotope ratios with GPS tracking in three gull species (lesser black-backed gull - *Larus fuscus*, great black-backed gulls - *Larus marinus* and European herring gull *Larus argentatus*) breeding in South-West France. Long-chain perfluoroalkyl carboxylates (PFCAs) were positively correlated to marine ($\delta^{13}\text{C}$ and $\delta^{34}\text{S}$) and high trophic level resources ($\delta^{15}\text{N}$) in herring gulls. This was confirmed by GPS tracking, with positive associations between PFAS concentrations and the proportion of the foraging sites located in the marine environment. In contrast, PFAS concentrations and stable isotope values were weakly and negatively associated in lesser black-backed gulls, with no link with the proportion of marine foraging sites, suggesting that other drivers were involved. For great black-backed gulls, only stable isotope ratios were quantified and were not associated with PFAS concentrations. These species-specific associations between PFAS and trophic ecology (isotopic markers and GPS) might stem from a contrasted use of the marine environment depending on the species, with terrestrial-coastal foraging habitats for herring gulls, terrestrial-coastal-pelagic foraging habitats for lesser black-backed gulls and only coastal for great black-backed gulls. Overall, the results suggest that the use of marine foraging habitats can increase exposure to PFCAs, particularly for species relying mostly on coastal areas. Mixed use of the marine environment (coastal/pelagic) and the potential pelagic dilution of PFAS may complicate our understanding of PFAS contamination, calling for further research. Combination of biologging with ecotoxicological study demonstrates that multidisciplinary approaches are essential to a comprehensive understanding of spatial exposure of wildlife to PFAS.

2.12.P-We159 Multispecies Contamination with Per- and Polyfluoroalkyl Substances: A Large-Scale Study in Metropolitan France and Sub-Antarctic and Antarctic Territories

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Per- and polyfluoroalkyl substances (PFAS) are persistent and ubiquitous chemicals whose effects on wildlife are of growing concern, requiring urgent large-scale monitoring. Seabirds are apex predators commonly used as bioindicators of marine pollution. The extent of PFAS contamination in seabirds is however still poorly known in many areas. Here we aimed at filling this gap by studying nine species from metropolitan France (M-Fr) and 15 species from French Southern and Antarctic Territories (TAAF), thereby providing insights into PFAS contamination in seabirds from temperate (M-Fr), subantarctic and Antarctic ecosystems. 11 PFAS were analysed in the plasma of chicks from M-Fr (n = 340) and the TAAF (n = 167). PFOS was the dominant PFAS, being detected in almost all chicks from both M-Fr (100%) and the TAAF (91%). In M-Fr, mean linear-PFOS concentrations ranged from 15.8 ± 6.6 ng g⁻¹ ww in Audouin's gulls to 77.4 ± 21.8 ng g⁻¹ ww in Scopoli's shearwaters. In the TAAF, linear-PFOS concentrations ranged from 0.7 ± 0.5 ng g⁻¹ ww in macaroni penguins (the least contaminated species) to 14.9 ± 4.6 ng g⁻¹ ww in giant petrels, with kelp gulls and Adélie penguins showing nonquantifiable concentrations (<0.2 ng g⁻¹ ww). PFNA, PFDA, PFUnDA, PFDoDA and PFHxS were also quantified in more than 70% of chicks in M-Fr, while these PFAS were only quantified in predatory and scavenging species in the TAAF (albatrosses, giant petrels and skuas). PFUnDA was the second most dominant PFAS, in both M-Fr (from 1.2 ± 0.6 in yellow-legged gulls to 9.4 ± 2.1 ng g⁻¹ ww in Scopoli's shearwaters) and the TAAF (from 0.7 ± 0.2 in snow petrels to 1.6 ± 1.3 ng g⁻¹ ww in brown skuas). Trophic ecology (foraging habitat and trophic position) studied via the stable isotope technique seemed to be a significant driver of carboxylic perfluoroalkyl acid concentrations at both the intra- and inter-specific levels in M-Fr. Overall, concentrations in the TAAF were low when compared to M-Fr, confirming a lower exposure in oceanic habitats of the Southern Hemisphere, far from direct emission sources. In M-Fr, 88% of chicks were above PFOS toxicity thresholds reported for avian model species, calling for further research into potential toxic effects of multi-PFAS contamination in early life stages.

2.12.P-We160 Organophosphate Esters in a Firn Core from Holtedahlfonna, Svalbard

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In spring 2018, we drilled an 11.6 meter-deep firn core from Holtedahlfonna, a large glacier on western Svalbard. The drill site is at 1150 m a.s.l. and the age at bottom of the core was 14.35 years. The core was cut into 9 sections to for minimum 500 mL sample size. The OPE analytes include 17 different compounds analyzed by LC-MS/MS. The OPEs detected in all samples included triethyl phosphate (TEP), tris(2-chloroethyl phosphate (TCEP), tris(2-chloroisopropyl) phosphate (TCIPP), triphenyl phosphate (TPP), tributyl phosphate (TnBP), tris(2-butoxyethyl phosphate (TBEP), 2-ethylhexyl-diphenyl phosphate (EHDPP). Tris(1,3-dichloro 2-propyl) phosphate (TDCPP) was detected only in the surface sample. The highest concentration in 7 of 9 samples was TCIPP ranging from 13.2 ng L⁻¹ to 143 ng L⁻¹ at the surface, with the other 2 highest being TBEP which throughout the core ranged from 9.34 ng L⁻¹ to 56.5 ng L⁻¹ again highest at the surface. The total OPE amounts detected ranged from 48.9 ng L⁻¹ at year 1995 to 265 ng L⁻¹ in the surface sample representing winter 2017-2018 accumulation, a factor of 5.4 increase. The percent chlorinated is 56.7 at the surface which is the only sample where the chlorinated fraction is greater than non-chlorinated in part because of the comparatively high amounts of TBEP throughout the core, except at the surface. This is unlike air data collected at Ny-Ålesund, Svalbard, (37 km southwest from Holtedahlfonna) in 2021 where chlorinated OPEs comprised 81 percent of all detected OPEs. In the Austfonna 2015 firn core drilled 230 km east from Holtedahlfonna at 700 m a.s.l., the percent chlorinated

ranged from 51 to 70 percent in six of 15 samples, suggesting that chlorinated compounds are deposited to glacier surfaces on Svalbard only with high atmospheric concentrations. TCIPP and TnBP at Holtedahlfonna are highly correlated with $r = 0.913$, significance < 0.05 suggesting that they were mixed when used at the source, similar to results for the Austfonna 2015 firn core.

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2.12.P-We161 Leveraging Mountain Pine Needles as Bioindicators of Potentially Toxic element Contamination in High-Elevation Ecosystems

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Pine needles from various species have long been utilized as bioindicators of air pollution. The accumulation of major and trace elements in these needles results from both dry and wet deposition of airborne pollutants, as well as the uptake of substances through plant roots. Environmental pollutants can be transported over long distances and reach remote areas. To investigate this, a mountainous region at the confluence of the Lower Inn Valley and Achenal in Tyrol, Austria, was selected for sampling. Needles from mountain pines (*Pinus mugo*) were collected at three different sites at altitudes from 1400 meters to 2000 meters. Sampling covered needles of varying ages young, one-year-old, two-year-old, and older from five to ten trees at each site, pooling needles of the same age. Sampling was conducted annually every July from 2018 to 2024. For elemental analysis, dried samples underwent acidic digestion (either microwave assisted or by high pressure asher) followed by quadrupole and high-resolution inductively coupled plasma mass spectrometry (ICP-MS) measurements. The metabolic state of the plant material was monitored in a fast and non-destructive way by Raman spectroscopy providing additional information on the growing conditions of the mountain pines. The highly sensitive differentiation in fingerprint region of the spectra allows the classification of differently grown pine needles. Rigorous quality assurance protocols were implemented to ensure the reliability of the analytical results and their comparability with previously published data. The findings revealed that altitude had a greater effect on the concentrations of macroelements than on potentially toxic elements. Like other pine species, age-related accumulation of certain elements was observed in the needles.

2.12.P-We162 Chemical Contaminants in Alpine High-Mountain Lakes: Sources, Transport Mechanisms, and Ecological Implications

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High-mountain lakes in the Alps, despite their remote locations, are highly susceptible to chemical pollution. This review highlights their role as reservoirs for Persistent Organic Pollutants (POPs) and Contaminants of Emerging Concern (CECs), examining their origins and the resulting environmental and human health implications. Regarding POPs, 14 studies have documented the occurrence of polychlorinated biphenyls, dichlorodiphenyltrichloroethane (DDT) and its metabolites, polybrominated diphenyl ethers, and polycyclic aromatic hydrocarbons in high-altitude Alpine lakes. Most of this research has focused on the Italian Alps (63%), followed by Switzerland (22%), Austria (12%), and France (3%). Sediments are the most frequently studied compartment (65%), with fish (33%) and water (2%) receiving less attention. Similarly, six studies have investigated the presence of CECs in these environments, including musks, perfluorinated compounds, and microplastics. Research on CECs is primarily concentrated in Switzerland (42%), France (33%), and Italy (25%), with fish tissues (muscle and liver) being the predominant focus (46%), followed by sediment (17%) and water (17%). Other compartments, such as zooplankton, amphibians (frogs and tadpoles), and snow, remain underexplored. The review also addresses the key pathways by which pollutants reach these remote ecosystems, including atmospheric deposition, glacial meltwater, and human activities. Safeguarding these fragile environments necessitates sustained research, comprehensive monitoring, and targeted conservation strategies.

2.12.P-We163 Toward a Sociological Approach of Emerging Pollutants (Microplastics, PFASs) Presences in the Alps

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Each year, recreational activities such as hiking, ski-touring or mountaineering bring more and more

people in contact with alpine ecosystem. Recently, sociology showed how these practices seem to be driven by practitioners' dispositions to feel attached and to perceive alpine abiotic elements, fauna or plants. However, in one hand, hikers tend to leave offscreen of consciousness what remind them of touristic harnessing and the component of their practices which contribute to mountain pollution (waste, uses of high-tech textiles, road traffic). In another hand, alpine park managers confront overtourism and question how they can increase public awareness about mountain ecosystem vulnerability. The threat. The presence of emerging pollutants in these remote areas, brought by practitioners and their equipment, represents a challenge for both social sciences and environmental sciences.

This study questions the historical and social constitution of emerging pollutants (microplastics, PFASs) as objects of scientific research in the Alps, i.e., how they have been put on the agenda of environmental sciences, or not, and how in return research contributes to specifying their presences. At the crossroads of sociology of science and sociology of the environment, this research focuses on the circulations and presence modes of these pollutants within mountainous areas. It aims to identify the knowledge produced and the scientific practices used to study these pollutants described as persistent in the context of climate transition.

Based on a survey combining bibliographic research, sociological interviews and ethnographic fieldwork, the objective of this study is (1) to reconstruct the sociohistorical context of the scientific management of persistent pollutants, particularly in the Alpine region, and (2) to question in situ how environmental science researchers respond to the environmental and health issues represented by PFAS and microplastics.

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2.12.P-We164 Two-Year Study of Microplastic Contamination and Ecological Risks in Fish Species of an Alpine Lake (Cesana Torinese, Northwest Italy)

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Despite their remote location, alpine lakes are highly vulnerable to environmental changes and pollution. These lakes are increasingly affected by various anthropogenic stressors, and contaminants such as microplastics (MPs) that can be transported through atmospheric deposition, runoff, and human activities. Alpine lakes are typically fishless due to their isolated locations and extreme conditions, though fish have occasionally been introduced for recreational fishing. Using Lake Nero in Cesana Torinese, Italy, as a case study, a two-year research project aimed to assess the prevalence, distribution, and potential ecological risks of MP contamination in fish species. This study explores the impacts of anthropogenic pollutants like MPs, even in remote alpine lakes with minimal human presence, but where activities such as recreational fishing occur.

Fish were sampled in the summers of 2023 and 2024 to assess MP contamination in their gastrointestinal tracts. MPs were analyzed for size, color, frequency, and polymer type using Fourier-transform infrared (FT-IR) spectroscopy, and comparisons were made between the two years. In 2023, MPs were predominantly found in *Salmo trutta*. Polymers such as polypropylene (PP), polyethylene terephthalate (PET), and polyethylene (PE) were identified, suggesting an origin from common consumer goods or degraded plastics in the environment. In 2024, sampling expanded to include *Salvelinus fontinalis*, which was absent in 2023, providing a broader assessment of MP variability in the lake.

The results suggest that MP contamination could pose ecological risks, including bioaccumulation in the food web and potential physical or chemical effects on aquatic species. A risk assessment conducted as part of the study emphasized the importance of monitoring MPs as emerging contaminants, even in remote ecosystems. This research highlights the need for conservation and mitigation strategies to protect alpine lakes and their fragile ecosystems.

2.12.P-We165 Ecotoxicological Effects of Potassium Chloride on *Daphnia middendorffiana* From Alpine Lake Under Warming Scenarios

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Climate change directly affects aquatic ecosystems by increasing water temperatures, which can lead to shifts in the distribution and range of aquatic species. Impacts will affect all biomes, with alpine environments being particularly vulnerable. This study investigates the combined effects of temperature increase and potassium chloride (KCl) exposure on *Daphnia middendorffiana*, an endemic species from high-mountain lakes, compared to *Daphnia magna* as a model organism.

Zooplankton sampling was carried out in the Upper Balma Lake (Northern Italy) for the research of *D. middendorffiana* while *D. magna* was obtained from MicroBioTest Inc. Specimens were exposed to varying concentrations of KCl for 24 and 48 hours at two temperatures: 15°C (lake temperature) and 20°C (simulating a warming scenario). Immobilization was measured as the endpoint to assess the ecotoxicological effects of KCl under different thermal conditions.

The results showed that both species showed a significant negative response to KCl exposure, with complete immobilization observed at higher concentrations. The synergistic effect of temperature on toxicity was evident in *D. middendorffiana*, as the EC values (effective concentrations) were consistently lower at 20°C, suggesting that warmer temperatures reduce the tolerance to KCl. These results are consistent with the hypothesis that temperature increases can exacerbate the toxic effects of pollutants, potentially disrupting the trophic dynamics of high-altitude lakes. Both daphnid species showed an enhanced effect with the co-exposure to a non-optimal water temperature (20 °C for *D. middendorffiana* and 15 °C for *D. magna*).

The study highlights the importance of considering both climate change and chemical pollution when assessing the risk of alpine ecosystems.

2.12.P-We166 Effects of Water Chemistry and Contaminants on Antioxidant Responses in *Cottus gobio* from a High-Mountain Lake

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Oxidative stress is a critical factor in fish ecology, influenced by both internal and external factors like metabolic processes, pollutants, and fluctuations in water conditions. High-altitude lakes, with their unique environmental conditions, can increase oxidative stress in fish due to low temperatures, high UV radiation, and nutrient scarcity. Monitoring oxidative stress markers in fish from these lakes provides valuable insights into their health and adaptation to extreme environments.

Dimon Lake, located at 1,857 meters in the Carnic Alps of northeastern Italy, is a glacial lake that hosts *Cottus gobio* as the only fish species. In 2021, thirty fish were sampled in both summer and autumn. Water quality was assessed, measuring temperature, dissolved oxygen, conductivity, pH, and concentrations of ammonia, nitrate, and phosphate. Additionally, fish muscle tissue was analysed for trace elements (As, Cd, Pb, Zn), PAHs, non-dioxin-like PCBs (NDL-PCBs), and pesticides. Microplastics were also examined in the fish gastrointestinal tracts. Oxidative stress biomarkers, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GR), and metallothioneins (MTs), were evaluated in liver, gill, and muscle tissues. Significant seasonal variations were observed in water parameters. In summer, oxygen, pH, conductivity, and phosphate levels were lower compared to autumn, while temperature, ammonium, and nitrate concentrations were higher in the warmer months. In fish muscle, metal concentrations were notably higher in autumn. PAHs, NDL-PCBs, and pesticides were below detection limits, and no microplastics were found. Biomarkers of oxidative stress revealed organ-specific and seasonal patterns. The liver exhibited the highest activities of different biomarkers, emphasizing its central role in detoxification. A significant correlation was found between pH and the activities of SOD, CAT, GPx, GR, and MTs, suggesting that water chemistry strongly influences oxidative stress responses. Elevated MT levels in autumn suggested increased exposure to metals or other environmental stressors, such as pH fluctuations.

This study highlights seasonal changes in water quality, contaminants, and oxidative stress in a high-mountain lake. Long-term research is needed to assess the impacts of climate change on these sensitive ecosystems and inform conservation strategies to ensure their resilience.

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2.12.P-We167 Comparison of Pollution in High Mountain Aquatic Ecosystems - Glacial Tarns and Alpine Stream and Effects of Flash Flood Using Alpine Bullhead as an Indicator

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The alpine bullhead (*Cottus poecilopus*) is representative of the ichthyofauna of high mountain stream and lake ecosystems in the Western Carpathians. This study aimed to investigate the spatio-temporal distribution and transport of mercury, zinc, molybdenum, rubidium, and strontium from alpine lake to alpine stream ecosystems and to compare elemental concentrations among populations of alpine bullhead in alpine tarns and mountain stream. Elemental analysis was performed on collected dead fish found in three tarns in the West Tatras (n = 15) and the Javorinka stream (n = 51) from 2017 to 2022. After all manual and instrumental measurements, the alpine bullheads were stored in freezers to preserve the biological material for the future. was used for fast and accurate measurement of element concentrations in fish bodies. Mercury was measured using a DMA-80 direct mercury analyzer (Milestone, Italy) for total mercury in dry weight. Concentrations of mercury and zinc in fish from the lake were found to be 6 and 2.5 times higher, respectively, than in fish from the stream. Rubidium, molybdenum, and strontium concentrations did not differ between lacustrine and fluvial fish, suggesting that their sedimentation patterns are similar in rivers and lakes. In July 2018, a major flood occurred in the area of the Javorinka stream. This year, the mercury content in alpine bullheads in the Javorinka stream increased significantly. Biomagnification of mercury in fish occurred very quickly after the flood and was significant also in the following year, 2019. Subsequently, there was a notable decline in mercury concentrations, reaching a reduction of up to 70% between 2021 and 2022. As this research continues over the next years, changes in other biogenic elements between the tarns and the stream may become apparent due to climate change, and because the tarns are more isolated ecosystems by the mountains. This may make the high mountain tarns even more unsustainable for any life in the future than they are now.

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2.12.P-We168 Seasonal Dynamics and Biota's Role in Elemental Transfer and in the Javorinka Mountain Stream Ecosystem

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The impacts of climate change are causing significant changes in aquatic biogeochemical processes and, through changes in the distribution and levels of elements, may alter the dynamics and quality of aquatic habitat biodiversity. Seasonal dynamics of elements are influenced by local environmental factors, seasonal influences and short-term events (droughts, floods). Assessing the connectivity and importance of elemental transfer through the food chain with respect to seasonality will allow for a more comprehensive assessment of water quality over time, helping to evaluate both current and potential constraints. Elements enter the cycle from basic natural sources and are taken up and transferred by organisms.

This study summarises the current knowledge on the accumulation of elements in individual food chain components (algae, macrozoobentos, fish) in the Javorinka mountain stream (High Tatras, Western Carpathians) through long-term monitoring (2019-2023) and analysis of elements in tissues using X-ray fluorescence spectroscopy (ED-XRF). In this study, we focused on the transfer of elements (S, Cl, K, Cr, Zn, Fe and Mn) in food chain components in cyanobacteria (*Oscillatoriales*), insect orders (Ephemeroptera, Plecoptera) and fish (*Cottus poecilopus*). We found that the highest levels of elements shifted between groups from lower to higher trophic chain levels mainly in the following seasons. The highest values of the elements S, Cl, K were recorded in cyanobacteria in spring, in invertebrate larvae in summer and in fish in winter. The elements Cr and Mn are highest in both cyanobacteria and insect larvae in autumn, and in fish in winter. Fe is highest in *Oscillatoriales* in autumn, in insect larvae in autumn and winter, and in fish only in spring. Zn is highest in *Oscillatoriales* in summer, but only in insect larvae and fish in winter. The uptake of elements by biota depends on their availability in the environment. Their excretion and transport is mainly influenced by dietary and metabolic patterns during ontogeny and biomagnification.

Mountain streams also play an important role in transporting sediment and nutrients to lower areas and are

a source of drinking water. Long-term studies of elemental dynamics in stream biota are important not only for understanding the processes occurring in streams, but also for assessing the impacts of climate change, such as flash floods, prolonged droughts or other extreme events that alter elemental cycling and levels.

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2.12.P-We169 Indication of Environmental Pollution by Mercury and Other Elements Using Equivalent Alpine and Subalpine Mammals from the Western Carpathians and the Zhongar Alatau
Lenka Zábajníková, Zuzana Kompisova Ballova, Martina Haas and Marian Janiga, Institute of High Mountain Biology, University of Žilina, Slovakia

Heavy metals and other potentially toxic elements accumulate in remote mountain areas through atmospheric transport. Previous studies have identified the Western Carpathians as one of the most heavily polluted mountain ranges. For comparison, we chose the Zhongar-Alatau mountain range in Central Asia, where pollution from the surrounding industrialised countries is also thought to be high. As herbivorous species are considered to be one of the best indicators of heavy metal pollution from the air, we decided to use samples from herbivorous mammals living in the alpine and subalpine areas of the Western Carpathians and the Zhongar Alatau. We collected non-invasive samples of marmot faeces and bones of naturally deceased ungulates. Marmots (*Marmota marmota latirostris*) and Tatra chamois (*Rupicapra rupicapra tatrica*) are symbols of Tatra National Park in the Western Carpathians, they are iconic species with high conservation value. For comparison, we chose ecological equivalents of these species from Zhongar Alatau (*Marmota baibacina*, Asiatic ibex, Marco Polo sheep, other Bovidae and horses). The samples were collected in autumn (September-October) 2022. The faecal samples were analysed by X-ray fluorescence for detection of S, Ba, Mn, Mo, Zn, Cu, and Cr. Mercury concentrations in faeces and bones were determined using a DMA-80 analyzer. We found that marmot faeces from the Western Carpathians contained significantly more amount of total mercury ($p < 0.001$; mean = $0.066 \mu\text{g/g}$ dry weight) than marmot faeces from Zhongar Alatau (mean = $0.034 \mu\text{g/g}$ dry weight), as well as sulphur and selected heavy metals (Ba, Mn, Mo, Zn, Cu, and Cr). Bones of ungulates found in Zhongar Alatau had significantly lower levels of total mercury ($p < 0.05$; mean = $0.0024 \mu\text{g/g}$ dry weight) than bones found in the Western Carpathians (mean = $0.0063 \mu\text{g/g}$ dry weight). The Western Carpathians are relatively more polluted by mercury, sulphur and heavy metals (Ba, Mn, Mo, Zn, Cu, and Cr) from anthropogenic activities than the Zhongar Alatau Mountains. The most likely sources of pollution are the continuing industrialisation of north-western Europe, mining and smelting, and traffic emissions, which can reach remote mountain ranges through long-range atmospheric transport by the westerly winds that prevail in this region.

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2.12.P-We170 Impact of Flash Floods on Physicochemical Dynamics in Alpine Streams: Insights from Long-Term Monitoring in the Tatra Mountains

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Physicochemical changes of water in alpine/mountain streams can provide useful information on ongoing natural and anthropogenic processes in their catchments. Periodical monitoring of these changes can determine the extent of seasonal variations or define repeating patterns (cycles) and mutual interactions. Particularly important in the context of ongoing climate change is observing the development of conditions following extreme or accidental events that can significantly affect dynamic trends in these vulnerable aquatic ecosystems.

Over five years of continual (weekly) monitoring of physicochemical conditions in the alpine/mountain stream Javorinka in the Tatra Mountains, we recorded one major flash flood (July 2018), which not only significantly altered the stream morphology but also affected the trajectories of organic and inorganic compounds. For example, we observed a significant decrease in the chemical consumption of oxygen and ammonia after the flood, alongside an increase in oxygen saturation and nitrate concentrations. Streambed erosion caused elevated phosphate levels over the subsequent two years and a marked enrichment of the water with dissolved solids during the following spring seasons.

We hypothesize that, from a long-term perspective, such flash floods are critical in sustaining the oligotrophic profile of alpine and mountain streams. To test this hypothesis, our main objective is to continue monitoring and compare the seasonal trends of key physicochemical parameters in the stream before and after such events. Based on paleohydrological studies, flash floods in the Tatra Mountains are

recurring natural phenomena, typically occurring every 6–9 years, and are expected to increase in frequency and severity due to climate change. Observing future flash floods will reveal whether these events continue to reinforce the regular patterns and interactions that maintain the oligotrophic characteristics of these ecosystems.

The impact of flash floods on the physicochemical dynamics of alpine streams is poorly understood. Understanding these effects is crucial for predicting long-term changes and provides insights into the resilience and dynamics of vulnerable mountain aquatic ecosystems.

Track 3. Environmental Chemistry and Exposure Assessment: Analysis, Monitoring, Fate and Modelling

3.01 Innovative Analytical Methodologies to Support Next-Generation Risk Assessment and Early Warning

3.01.T-01 Innovative Suspect and Non-Targeted Screening Approaches as a Support to Chemical Risk Assessment: From Research Results and Promises to Regulatory Expectations

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Our environment is a vector of exposure to thousands of chemicals, some of which may be harmful for ecosystems and humans. This complexity of real-life chemical exposure is a major challenge for the scientific community and public health authorities. Suspect and non-targeted screening approaches (SS/NTS) have emerged to expand our knowledge on this environmental and human chemical exposure. These approaches have been a matter of various research based proof-of-concepts in the environment, food and human biomonitoring fields, illustrating their potential and generating high expectations for support to policy purposes. This last application area is however associated to a number of challenges requiring to clarify what could be and could not be expected from these approaches. This work aims at presenting a number of applications of SS/NTS to environmental and human sample specimen, based on results originated from research program conducted at EU level. Based on these illustrating examples, a clarification regarding their positioning for risk assessment is given.

In the Human Biomonitoring for Europe initiative (HBM4EU, H2020, 2017-2022), a number of methodological development and harmonisation actions were conducted with regard to SS/NTS approaches applied to human samples. A summary of the elaborated QA/QC provisions for data generation and result reporting will be given. The results of a wide multicentric study (SPECIMEn) consisting to analyse 2000 human urine samples collected in 5 different EU countries by 5 different EU laboratories thanks to a SS approach under harmonized conditions will be then presented.

In the Partnership for the Assessment of Risks from Chemicals (PARC, Horizon EU, 2022-2029), the methodological improvement and harmonization objective regarding SS/NTS approaches is pursued in a more integrated perspective across the environment-food safety-human biomonitoring silos. A summary of the established worklines for harmonisation will be given, as well as first results of real sample analyses.

In the project Providing risk assessments of complex real-life mixtures for the protection of Europe's citizens and the environment (PANORAMIX, H2020 Green Deal, 2021-2025), a SS profiling approach was applied to reveal, compare and connect real-life chemical mixtures present within an environmental-food-human continuum. The applied analytical strategy from sample preparation to data analysis, as well as main results obtained will be described.

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3.01.T-02 Utilities of Long-Term Chemical Pollution Monitoring Data of Surface Waters in the Netherlands

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Chemical pollution is considered as one of three planetary crises. Long-term chemical pollution monitoring data for an area provide information to characterize spatial patterns and temporal trends, and to define regulatory measures to prevent and limit the pollution. Such data are however commonly dispersed over various sources and difficult to obtain and use in practice. This research aimed to collate spatio-temporal monitoring data, to support developing or validating novel risk characterisation models and mitigation approaches.

This research collated data on chemical pollutants in Dutch surface waters covering the whole country in a 68-year time period till 2020 measuring 1712 parameters. Data were harmonized to provide a resource for scientific research and practical purposes in characterization, prevention and mitigation of chemical pollution of surface waters. The collated data set contains a total of 42.5 million spatiotemporal measured water quality parameters, representing the most dense water quality set of Europe, available for the evaluation of the water quality goals of the Water Framework Directive in 2027, the development of

exposure models, and developing risk assessment and mitigation approaches.

The harmonization of the data collated by different waterboards, while crucial for spatiotemporal district comparison, highlighted the need for more integrated approach. Differences in units, measurement techniques and the decimals/accuracy reported between waterboards and over, lower the quality of an otherwise very rich dataset. Nevertheless, the dataset allows for the analysis and characterization of both spatial and temporal trends. The dataset has been used to show mixture toxic pressure has high spatial variability. Moreover, the management efforts that were taken to lower the level of imidacloprid in an area have been effective in lowering the concentration over time, as is shown by the data.

This research shows that collated monitoring results are a rich source to support risk assessment and management of pollutants. The concentration data are useful to develop and improve region-specific exposure models, able to prevent cumulation of risk beyond the no-effect level. Moreover, the dataset may be combined with hazard data, to derive spatial and temporal patterns in impacts of chemical pollution on aquatic life. These approaches together are supportive of next generation risk assessment methods, and could result in an early-warning approach.

3.01.T-03 In Vitro Bioassay Profiling of Endocrine Activities in European Surface Waters: A Large-Scale Pilot Project

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Endocrine-disrupting compounds (EDCs) are structurally diverse chemicals from various sources that can harm the environment and human health. Monitoring EDCs with chemical methods remains challenging due to the vast number of potential compounds to be assessed at low concentrations in complex environmental matrices. Effect-based monitoring using in vitro bioassays has been proven as a relevant tool to reveal the presence of EDCs in a sample, but without identifying the responsible compounds. Thus, combining bioassays with chemical analysis offers a promising approach for the detection and identification of EDCs, with a view to tracing their sources, and understanding their biological effects. Within the Horizon Europe Partnership for the Assessment of Risks from Chemicals (PARC), monitoring strategies integrating both chemical and effect-based methods have been developed to characterize EDCs in air, soil, surface water, and biota. This presentation will focus on effect-based assessment of EDCs in European surface waters using a comprehensive panel of in vitro bioassays.

Sampling campaigns were conducted in 2024 by collaborative efforts of multiple laboratories, covering different regions in Europe and types of anthropogenic pressures (industrial and agricultural regions, urban run-off, and waters receiving effluents from wastewater treatment plants). A total of 117 water samples were collected and processed following standardized extraction procedures. The organic extracts were then subjected to in vitro receptor-based bioassays targeting 10 human nuclear receptors (AR, ER, GR, MR, PR, AhR, PXR, PPAR, RAR/RXR) and the thyroid hormone binding protein TTR. In addition, for some modes of action with cross-species differences, both human and zebrafish receptors were employed to reveal possible effects specific to aquatic vertebrates. Furthermore, quality assurance and controls were defined and implemented for sampling, sample preparation, and bioassays.

Bioactivity profiles observed in the water samples will be presented and discussed in terms of the type of endocrine receptor activated and site typology. Potential differences between human and zebrafish bioassay responses will be reported and discussed regarding an EDC monitoring strategy. In the future, bioassay results will be compared with target chemical analyses to assess the contribution of prioritized EDCs to the observed bioactivity.

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3.01.T-04 Integration of Dysregulation Measures in Behavior and Exo-Metabolic Profile for Biological Early Warning System Using *Daphnia magna*

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Biological early warning systems (BEWSs) utilize organismal responses to pollutants for continuous water quality monitoring and detection of a wide range of toxic substances. *Daphnia magna* alters its swimming behavior in response to chemical or environmental stress, making it a valuable bioindicator. However, the reliability of *Daphnia*-based BEWS is often challenged by false alarms due to non-specific behavioral responses. Beyond behavioral monitoring, recent research explores metabolomics to provide a complementary perspective in water quality assessment. This study aims to enhance BEWS by integrating behavioral responses of *D. magna* with exo-metabolome analysis. Using high-throughput video tracking and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS), a time-course analysis was conducted to examine locomotion and metabolic regulation under various exposure conditions.

Among 1,228 metabolic features, 841 features were significantly increased in the group containing *D. magna* compared to the control group containing only river water (false discovery rate (FDR) adjusted $p < 0.05$). Of these, 487 features displayed significant differences across pre-exposure (Pre), exposure (Exp), and post-exposure (Post) conditions ($p < 0.05$). Metabolic features exhibiting continuous increases or decreases across Pre, Exp, and Post groups were identified as potential markers for chemical exposure. During copper exposure, metabolic features correlated with copper concentration were observed. Among the 487 significant features, 28 showed a decreasing trend, and 3 exhibited an increasing trend ($p < 0.05$). In this study, annotation was not possible due to the lack of an exo-metabolome database and derivatization, and the functions of the detected features could not be confirmed. In behavioral analysis, we expect that the average swimming speed, swimming angle, and dispersion of *D. magna* will alter more rapidly at higher copper concentrations.

This study demonstrates the potential of integrating exo-metabolomic data with traditional behavioral monitoring in *D. magna*-based BEWS. Future research will focus on validating the reproducibility of these findings in diverse river waters with varying physicochemical properties. This integrated approach offers a promising avenue for advancing BEWS, ensuring more accurate and timely detection of water quality changes while reducing false alarms.

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3.01.P-Tu197 A New Systems Approach Addressing Chemical Risks to Terrestrial Biodiversity in Europe

Guy Duke¹, Nikiforos Alygizakis¹, Paola Movalli², Susan Oginah³, Gabriele Treu⁴, Nikolaos S. Thomaidis⁵, Marissa B Kosnik⁶, Martina G. Vijver⁷, Henrik Barmentlo⁷, Koos Biesmeijer², Peter Fantke¹, Georgios Gkotsis⁵, Romana Hornek-Gausterer⁸, Olivier Jolliet³, Oliver Machate⁴, Peter Oswald¹, Laura Scherer⁷ and Jaroslav Slobodnik¹, (1)Environmental Institute, Slovakia, (2)Naturalis Biodiversity Center, Netherlands, (3)Technical University of Denmark, Denmark, (4)German Environment Agency (UBA), Germany, (5)National and Kapodistrian University of Athens, Greece, (6)Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland, (7)Institute of Environmental Sciences (CML), Leiden University, Netherlands, (8)Environment Agency Austria, Austria

Anthropogenic chemicals (with other novel entities) have passed the safe operating space of the planetary boundary, since annual production and releases outstrip global capacity for risk assessment and environmental monitoring. In Europe, the number of chemicals in use far exceeds capacities of conventional risk assessment and monitoring, chemical registration data is often inadequate and risk assessment does not sufficiently consider damages to biodiversity and ecosystem services, in particular in the terrestrial compartment. To address these challenges, we propose a new systems approach, which can support regulators in accelerating delivery of the European Union's zero pollution ambition in the terrestrial compartment. This approach is being developed and tested by the Horizon Europe project TerraChem. The systems approach integrates four key elements: (1) periodic pan-European monitoring of chemicals exposure and mixture effects in selected apex species food chains across representative terrestrial biomes; (2) modelling of chemical source-to-damage pathways in terrestrial ecosystems; (3) novel tools to prioritise substances for further risk assessment and novel approaches to better address terrestrial biodiversity and ecosystem services in environmental risk assessment and risk management of chemicals; (4) a European data management and early warning system for chemicals in terrestrial biota and soils that integrates the monitoring data, modelling output and prioritisation tools. This TerraChem approach would provide a near real-time picture of which substances among the entire universe of ERACs are turning up in terrestrial biota and soils across Europe, provide early warning of emerging contaminants, prioritise substances for further risk assessment and risk management measures, and enable

risk assessment and management to better take account of damages to terrestrial biodiversity and ecosystem services. TerraChem will test this systems approach, deliver a blueprint for a robust, coherent, pan-European monitoring-modelling-prioritisation-risk assessment and management scheme for chemicals in the terrestrial compartment, and identify next steps to put such a scheme in place. This platform presentation will present interim findings at the project s midway point.

Disclaimer/Disclosure: This work is funded by the European Union. Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

3.01.P-Tu208 Increased (S)VOC Identification in Complex Samples Using a Dual-Ionization EI&CI-TOFMS Hyphenated with a Flow Modulated GCxGC System

Sonja Klee¹, Steffen Braekling¹, Eliška Ceznerová¹, Marleen Vetter¹, Ralf Kurtenbach², Scott J. Campbell³, John Moncur³ and Arnd Ingendoh⁴, (1)TOFWERK AG, Switzerland, (2)University of Wuppertal, Germany, (3)SpectralWorks, United Kingdom, (4)Bruker Daltonics GmbH & Co KG, Germany

Hyphenation to GCxGC sets high requirements to a mass spectrometric detector. Narrow chromatographic peak widths require high mass spectral acquisition rates that are commonly achievable by EI-TOF instrumentation. Flow modulation GCxGC simplifies the experimental setup but also increases the demands to the MS due to enlarged column flows, resulting in high split ratios preceding the detector. However, when measuring complex samples or unknowns compounds an additional soft ionization technique, such as CI improves the compound identification process. For one dimensional GC the combination of EI and CI operating simultaneously on one analyzer within one single chromatographic run has been presented previously. In this study we show the combability and advantages of a fast-switching EI&CI-TOFMS coupled to flow modulation GCxGC.

Ambient air and a standard gas mixture were collected on desorption-tubes. The collected samples were desorbed, preconcentrated and injected into the GCxGC system including a flow-modulation setup. A standard GCxGC-method was provided by the collaborator. The EI&CI-TOFMS operating CI- and EI was used as detector to provide simultaneous molecular (CI) and structural (EI) information.

The hyphenation of a dual ionization EI&CI-TOFMS with a flow modulated GCxGC setup is presented. Special focus is set on: (1) characterization of the alignment of simultaneously generated EI and CI information; (2) the EI/CI switching behavior of the MS; (3) the chromatographic performance of the ionization sources; and (4) the advantages of using flow modulation GCxGC in combination with medium pressure CI sources. The complexity of the GCxGC-dual ionization MS coupling is addressed and experiments with the aim to harness the high gas flows used in flow modulation GCxGC were conducted. It can be shown that the high pumping efficiency in the CI interface can be utilized to increase the CI sensitivity by a factor of 30, by guiding the larger share of the flow ratio into the CI source. Using a specifically defined flow splitting, it was possible to maintain the flow balance within the EI pumping stage and simultaneously increase the CI performance leading to increased identification certainties as well as decreased limits of detection (LOD). The findings on the instrumental performance investigated via standard gas mixture measurements were further verified on ambient air samples collected at a federal highway in Germany.

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3.01.P-Tu214 Comprehensive Pesticide Screening with Dual Ionization GC-HRMS: Bridging Targeted and Non-Targeted Analysis

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The detection and quantification of pesticide residues and contaminants in agricultural products are critical for ensuring food safety and compliance with global regulations. The growing demand for these requirements, driven by consumer awareness and stringent regulations, increases the demands on analytical screening. Chromatographic techniques coupled to high-resolution mass spectrometry (HRMS)

are becoming a most reliable analytical platforms for pesticides analysis, mainly due to their comprehensive detection, and identification of both, known and unknown residues with high sensitivity and specificity. Here, we present a new non-targeted analysis approach of volatile pesticide residues in food matrices. For unambiguous compound identification a novel gas chromatography (GC)-HRMS system generating simultaneously EI and CI information on the detected analytes was utilized.

Agricultural products were extracted using QuEChERS protocols and analyzed with a GC 8500 system (Bruker, Germany) coupled to an ecTOF detector (Bruker, Germany). The system's dual ionization capability, combining electron ionization (EI) and chemical ionization (CI) simultaneously, provides complementary structural and molecular data. CI reagent ions (N_2H^+ , H_3O^+ , NH_4^+) were used to adjust fragmentation and enhance molecular detection. A targeted library of 213 pesticides was created for targeted analysis. Data post-processing for targeted, suspect and non-targeted approaches were carried out with AnalyzerPro XD (Spectralworks, UK).

The GC-HRMS system effectively identified pesticide residues with certainties above 70% for both EI and CI traces using the created targeted library. Non-targeted analysis detected additional compounds that were analyzed using NIST library searches and accurate mass data. Added CI information improved sensitivity and enabled residue detection at low ppb levels. The dual-ionization approach enhanced compound identification certainties, reduced analysis time, and increased reliability for complex samples. The integration of EI and CI in a single GC-HRMS workflow enhances certainties and reliability for both targeted and non-targeted pesticide residue analysis. The system shows to be a highly efficient tool for ensuring food safety and regulatory compliance, offering robust solutions for identifying pesticides in complex agricultural products.

3.01.P Innovative Analytical Methodologies to Support Next-Generation Risk Assessment and Early Warning

3.01.P-Tu196 Safe and Sustainable by Design Framework for the Next Generation of Wood Plastic Composites for Automotive Industry

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In recent years, the design and development of materials with advanced properties and reduced environmental impact has become a priority in different industry sectors. Achieving these objectives requires a holistic approach that integrates different advanced models addressing the materials and chemicals safety following the Safe and Sustainable by Design (SSbD) principles. This poster presentation shows how SSbD principles for safe and sustainable design can be applied to the use case of novel Wood Plastic Composites, which are developed as interior material for the automotive industry. Increased safety through reduced emissions of Volatile Organic Compounds (VOCs), reduced environmental impact and improved circularity of the developed materials are points addressed. On the one hand, it focuses on the development of renewable composites formulations with sufficient mechanical performance, considering thereby recycled polypropylene or polypropylene made from renewable resources as thermoplastic matrix in combination with micro-fibrillated cellulose and wooden fibers as a green alternative for lightweight materials to reduce dependence on oil-based materials and conflict mineral fillers and to increase material circularity. Since the addressed interior trims in the car interact with passengers in a relatively close environment, it is important to ensure that they are not a risk to human safety and health. Thus, novel composite materials based on either renewable and/or recycled plastic reinforced with cellulose and wood-based fillers will be investigated, covering emissions and hazard assessments to enable safe use of such materials from the early R&D phase without any impact on passengers. This work will also present the method development for rapid VOC screening by coupling of micro and emission test chambers with Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (VOCUS CI-TOF PTR/AIM) to analyze novel Wood Plastic Composites (WPCs).

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3.01.P-Tu197 A New Systems Approach Addressing Chemical Risks to Terrestrial Biodiversity in Europe

Guy Duke¹, Nikiforos Alygizakis¹, Paola Movalli², Susan Oginah³, Gabriele Treu⁴, Nikolaos S. Thomaidis⁵, Marissa B Kosnik⁶, Martina G. Vijver⁷, Henrik Barmentlo⁷, Koos Biesmeijer², Peter Fantke¹,

Georgios Gkotsis⁵, Romana Hornek-Gausterer⁸, Olivier Jolliet³, Oliver Machate⁴, Peter Oswald¹, Laura Scherer⁷ and Jaroslav Slobodnik¹, (1)Environmental Institute, Slovakia, (2)Naturalis Biodiversity Center, Netherlands, (3)Technical University of Denmark, Denmark, (4)German Environment Agency (UBA), Germany, (5)National and Kapodistrian University of Athens, Greece, (6)Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland, (7)Institute of Environmental Sciences (CML), Leiden University, Netherlands, (8)Environment Agency Austria, Austria

Anthropogenic chemicals (with other novel entities) have passed the safe operating space of the planetary boundary, since annual production and releases outstrip global capacity for risk assessment and environmental monitoring. In Europe, the number of chemicals in use far exceeds capacities of conventional risk assessment and monitoring, chemical registration data is often inadequate and risk assessment does not sufficiently consider damages to biodiversity and ecosystem services, in particular in the terrestrial compartment. To address these challenges, we propose a new systems approach, which can support regulators in accelerating delivery of the European Union's zero pollution ambition in the terrestrial compartment. This approach is being developed and tested by the Horizon Europe project TerraChem. The systems approach integrates four key elements: (1) periodic pan-European monitoring of chemicals exposure and mixture effects in selected apex species food chains across representative terrestrial biomes; (2) modelling of chemical source-to-damage pathways in terrestrial ecosystems; (3) novel tools to prioritise substances for further risk assessment and novel approaches to better address terrestrial biodiversity and ecosystem services in environmental risk assessment and risk management of chemicals; (4) a European data management and early warning system for chemicals in terrestrial biota and soils that integrates the monitoring data, modelling output and prioritisation tools. This TerraChem approach would provide a near real-time picture of which substances among the entire universe of ERACs are turning up in terrestrial biota and soils across Europe, provide early warning of emerging contaminants, prioritise substances for further risk assessment and risk management measures, and enable risk assessment and management to better take account of damages to terrestrial biodiversity and ecosystem services. TerraChem will test this systems approach, deliver a blueprint for a robust, coherent, pan-European monitoring-modelling-prioritisation-risk assessment and management scheme for chemicals in the terrestrial compartment, and identify next steps to put such a scheme in place. This platform presentation will present interim findings at the project's midway point.

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3.01.P-Tu198 Integrated Solutions for Managing Contaminants of Emerging Concern: TerraChem's Approach to Data, Modeling, and Visualization in Terrestrial Ecosystems

Nikiforos Alygizakis¹, **Guy Duke¹**, Paola Movalli², Susan Oginah³, Gabriele Treu⁴, Nikolaos S. Thomaidis⁵, Marissa B Kosnik⁶, Martina G. Vijver⁷, Henrik Barmantlo⁷, Koos Biesmeijer², Peter Fantke¹, Georgios Gkotsis⁸, Romana Hornek-Gausterer⁹, Olivier Jolliet³, Oliver Machate⁴, Peter Oswald¹, Laura Scherer⁷ and Jaroslav Slobodnik¹, (1)Environmental Institute s.r.o., Slovakia, (2)Naturalis Biodiversity Center, Netherlands, (3)Technical University of Denmark, Denmark, (4)German Environment Agency (UBA), Germany, (5)National and Kapodistrian University of Athens, Greece, (6)Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland, (7)Institute of Environmental Sciences (CML), Leiden University, Netherlands, (8)National and Kapodistrian University of Athens, Greece, (9)Environment Agency Austria, Austria

The TerraChem project addresses the pressing need for advanced monitoring and risk assessment of contaminants of emerging concern (CECs) in terrestrial ecosystems. Built on the NORMAN Database System and expanded through LIFE APEX and TerraChem innovations, the TerraChem Data Management System (TDMS) integrates tools like the Chemical Occurrence Database and the Digital Sample Freezing Platform (DSFP) to provide geo-referenced data and enable retrospective chemical screening. Following FAIR principles, TDMS connects with DiSSCo to bridge chemical and ecological data for enhanced risk evaluations. The TerraChem Early Warning System (TEWS), powered through APIs from the Chemical Occurrence Database and DSFP, and a user-friendly Dashboard translate data into actionable insights, supporting stakeholders in mitigating biodiversity loss through predictive modeling and advanced visualization tools. By identifying data gaps and providing benchmark values across Europe, TerraChem empowers decision-makers to prioritize monitoring and interventions. This integrated platform sets a new standard for chemical risk management in terrestrial ecosystems.

Disclaimer/Disclosure: This work is funded by the European Union. Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

3.01.P-Tu199 Enhanced EASZY Assay for a Comprehensive In Vivo Endocrine Activity and Toxicity Assessment of Environmental Samples

Alizee Desrousseaux, Benjamin Piccini, Valentin Bourges, Elise Chatillon, Abd-El-Rahman El-Mais, Nathalie Hinfrey, Selim Ait-Aissa and Francois Brion, INERIS, France

The development of specific effect-based method using in vivo bioassays to analyse complex mixtures is essential to monitor chemical pollution in the aquatic environment. Zebrafish embryo is a powerful and efficient model for such assessment due to its rapid development, transparency, and high sensitivity to a wide range of pollutants.

The standard OECD TG 250 EASZY initially designed for quantitative assessment of estrogenic activity has been enhanced by incorporating additional endpoints such as endogenous gene expression, dioxin-like activity measurements as well as developmental, sublethal and acute toxicity. This new protocol is specifically developed to maximize collection of information on in vivo impacts of environmental samples by optimising the use of embryo, while minimizing the volume of organic extracts.

Embryos are exposed in triplicate to five dilutions of organic extracts for 96 hours with daily medium renewal. Observations of developmental markers (e.g., hatching, oedema, bleeding, scoliosis) are monitored daily to assess potential developmental toxicity of environmental extracts. After 96 hours, embryos are allocated for in vivo imaging of GFP expression driven by the ER-regulated *cyp19a1b* gene to assess estrogenic activity, or processed for CYP1A enzyme activity measurement (EROD) to evaluate dioxin-like effects. Furthermore, embryos are preserved in RNAlater for subsequent gene expression analysis, focusing on key genes of the endocrine system such as *cyp3a65* involved in intestinal metabolism regulation, *cyp11c1* and *fkbp5* involved in GR signalling pathways and hypothalamo-pituitary interrenal axis.

The validation of this protocol, using reference molecules to assess the activity of estrogenic, dioxin-like, and glucocorticoid compounds, will be presented. The protocol will then be applied within the framework of the European PARC project to surface water samples under various anthropogenic pressures. The results will be discussed in relation to in vitro endocrine and chemical analyses performed on the same samples (see abstract by Châtillon et al. submitted for SETAC 2025).

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3.01.P-Tu200 High Throughput Adaptation of Standardized Ecotoxicological Tests for the Model Freshwater Invertebrate *Daphnia magna*

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Current environmental regulations require the standardized testing of chronic ecotoxicological endpoints as part of its approval processes testing, however such tests tend to be resource intensive, and new high-throughput methods are not favoured due to their lack of standardization. To address this, a high throughput experiment was designed by culturing a cohort of >200 *Daphnia magna* individuals in well plates, with the conditions specified in OECD test number 211. Five concentrations of eight chemicals with different modes of action (Polar narcosis and nAChR inhibitors) were tested over a period of 21 days, tracking growth with a spheroid counter and reproduction in the well plates. High survival was observed in controls and low concentrations for all chemicals. Despite variations in average observed size of the animals are due to their position during the scans, results show a clear dose-response effect for growth and reproduction, allowing for calculation of LOEC, NOEC and EC50. The results of this experiment demonstrate the capacity to acquire a large volume of data for chronic endpoints throughout time, while following OECD standards.

3.01.P-Tu201 Make Your Research Regulatory Relevant: How Scientists Can Contribute to Validated and Standardised Test Methods

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(NIA), Belgium, (5)UK Health Security Agency (UKHSA), United Kingdom, (6)UK Centre for Ecology & Hydrology (UKCEH), United Kingdom

New scientific developments of methods and materials are essential contributions to approach major global challenges. The development of new materials as well as the development of new safety test methods triggers the development of harmonised standards and OECD Test Guidelines. Up-to-date test methods support innovation, reduce trade barriers and testing efforts, save resources and enable international comparison.

Despite the urgent need for regulatory relevant test methods, good science does not automatically get used in regulation. Indeed, to be integrated in regulatory oriented standards or OECD Test Guidelines, new testing methods must be validated and standardised. This involves following specific agreed processes, which define timelines and requirements. These processes are often seen as complex or time and resources consuming by the scientific community, which inhibits method developers from translating their scientific methods and protocols into standards or OECD Test Guidelines.

Numerous incentives exist for scientists to be (more) active in the standardisation process and allow regulation to keep up with new scientific developments. These include an increase in research impacts, an expansion and diversification of the international expert network, increase of citations, ideation of new research questions and access to more fundings. Our work provides guidance on how to navigate successfully through the standards and OECD Test Guidelines development process, especially including requirements for method validation, which is a common prerequisite across the different standardisation bodies. We established in the EU project NanoHarmony a freely available set of resources for scientists in the OECD Test Guideline Process Mentor (<https://testguideline-development.org/>).

As scientific sound test methods and data are the core of OECD Test Guidelines and standards, a scientist can contribute in various different ways. Key aspects to consider in order to be successful with the development of standards and OECD Test Guidelines are to understand the main aspects of validation studies, to be in contact with the diversity of stakeholders acting in standardisation or harmonisation and to follow the defined steps and deadlines of the development process. The active participation of scientists along the entire process towards standards and OECD Test Guidelines is key for the transfer of the method into a wider, regulatory application and towards a safer world.

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3.01.P-Tu202 Tailored Analytical Approaches for Safety Assessment

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To achieve the goal towards climate neutrality and a toxic-free environment, the development of novel materials that are safe for humans and the environment, while also being economically sustainable is needed. Therefore, safe and sustainable by design (SSbD) voluntary approach, introduced in a 2022 Commission Recommendation, aims to guide the creation of safer and more sustainable chemicals and materials throughout their life cycle.

A critical aspect of the SSbD framework is the safety assessment of new materials, which involves identifying, quantifying, and assessing the risks associated with these materials through their life cycle. Tailored analytical approaches are therefore crucial in this process, providing essential data for hazard classification and predicting environmental behavior of the materials.

The main objective of this study is to evaluate different analytical techniques for conducting safety assessments of new materials in the textile industry during the product life cycle. To achieve this, a set of different analytical methods such as Pyrolysis Chromatography/Mass Spectrometry (Pyr-GC-MS), Dynamic Light Scattering (DLS), Nanoparticle Tracking Analysis (NTA), Atomic Force Microscope (AFM), and Scanning Electron Microscopy (SEM) will be presented.

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3.01.P-Tu203 Determination of Technology-Critical Elements in Urban Plants and Water using Inductively Coupled Plasma Tandem Mass Spectrometry

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Technology-critical elements (TCEs), e.g., Ge, Nb, Sb and the rare-earth elements (REEs), represent a non-uniformly defined group of elements that have become indispensable in various high-tech applications due to their unique chemical properties. Despite their rising use and documented instances of alterations of environmental background, the exact scope of anthropogenic releases, as well as their potential effects on ecosystems and human health, remain insufficiently characterised.

Regarding the environmental analysis of TCEs, challenges are posed by their chemical diversity, low environmental abundance, limited availability of (certified) reference materials, and spectral interferences encountered in inductively coupled plasma mass spectrometry (ICP-MS). This work addresses these challenges through the development and validation of a fast and robust measurement procedure using closed-vessel microwave-assisted acid digestion and ICP-tandem mass spectrometry (MS/MS). This validated method was employed to analyse urban environmental samples from Vienna, Austria, including plants from green facades and water from the Wienfluss river. The resulting data offers the first comprehensive assessment of certain scarcely studied TCEs in this context.

Key findings reveal patterns in the distribution of some TCEs that correlate with factors such as plant species, seasonality, and vertical positioning within the sampled sites. This work not only delivers a novel analytical approach for assessing TCEs but also presents an extensive dataset for understanding their distribution in a central European urban environment. The generated data lays the groundwork for assessments of potential ecological and human health impacts posed by anthropogenic TCE emissions.

3.01.P-Tu204 Enhancing Industrial Wastewater Monitoring: A Novel Dual Ionization GC-EI&CI HRTOFMS Approach for Comprehensive Pollutant Identification

Lucie Katharina Tintrop¹, Marleen Vetter², **Eliška Ceznerová²**, Sonja Klee², Steffen Brakling², Arnd Ingendoh³ and Thorsten Schmidt¹, (1)University of Duisburg-Essen, Germany, (2)TOFWERK AG, Switzerland, (3)Bruker Daltonics GmbH & Co KG, Germany

Wastewater treatment plants discharge effluents that often contain hazardous pollutants, posing significant risks to ecosystems and public health. Due to the highly variable composition of wastewater, routine monitoring with both targeted and non-targeted analyses is essential. Gas chromatography-mass spectrometry (GC-MS) is often combined with liquid chromatography-mass spectrometry (LC-MS) to expand the range of detectable compounds, enabling a more comprehensive assessment of potential hazards. However, conventional GC-EI-MS has limitations especially for non-targeted approaches, including low specificity in fragmentation patterns, missing molecular ion signals, and the absence of compounds in reference libraries, which all reduce the reliability of compound identification.

Industrial wastewater effluents were collected over a two months period using 24-hour composite samples. Analytes were enriched using a novel hydrophilic-lipophilic balanced (HLB) fiber coating (CTC Analytics AG, Zwingen, Switzerland) for headspace solid-phase microextraction (SPME). Analysis was performed with an Agilent GC (7890A GC, Santa Carla, California, USA) coupled to a dual ionization high-resolution mass spectrometer (ecTOF, Bruker, Bremen, Germany). This system enables the simultaneous acquisition of standard 70 eV electron ionization (EI) and medium-pressure chemical ionization (CI) (GC-EI&CI HRTOFMS) spectra in a single GC run. Flexible selection of CI reagent ions, such as [N₂H]⁺, [H₃O]⁺, and [NH₄]⁺ between different GC runs, enables adjustment of reactant selectivity and the degree of fragmentation for different analytes. This way the evidence generation for efficient compound

identification can be optimized.

Various strategies were employed to identify unknown compounds using EI and CI mass spectra acquired simultaneously. It is shown that incorporating different CI reagent ions significantly enhanced compound identification accuracy and confidence. Additionally, the novel HLB-SPME fiber enabled solvent-free headspace extraction and captures a broad spectrum of analytes. Combined with the GC-EI&CI HRTOFMS system, this approach demonstrates a high potential for detecting unknown hazardous compounds to advance industrial wastewater monitoring.

3.01.P-Tu205 Development of a Single Biofilm Extraction Method for Non-Target Analysis and Bioassays to Monitor Wastewater Micropollutants

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Passive samplers (PS) are strong tools to monitor micropollutants due to their ability to accumulate and concentrate the pollutants present in water. Among existing PS, those using biofilms as a receiving phase have gained interest for environmental monitoring, notably in waste water, for which conventional PS are limited by biofouling. Extraction and (bio)analysis of contaminants adsorbed in biofilms still need optimisation in monitoring context. Non-target analysis has been increasingly used during the last decade to detect a large range of water micropollutants, including emerging contaminants, which positions it as a great tool for environmental monitoring. However, this method does not account for the biological activities of the compounds, and the impact of mixture effects on their activity. Hence, as a complementary approach, in vitro bioassays provide a global bioactivity profile of the water samples while considering all the bio-active micropollutants and their potential mixture effect. The combination of PS with bioassays and chemical analysis has already shown its effectiveness in characterizing water. This work aims to develop an approach based on the coupling of an innovative biofilm-based PS with non-target screening and in vitro bioassays to characterize wastewater. This presentation will mainly focus on the development a single biofilm extraction method for both non-target analysis and bioassays, allowing us to have a robust correlation between the compounds analysed and the activity measured. Several solvents, extraction methods, and clean-up strategies were implemented and compared for biofilm extraction. The extracts were then subjected to chemical analysis and in vitro bioassays. For the chemical analysis performed on a liquid chromatography coupled to a high-resolution mass spectrometry, the extraction efficiency was evaluated based on characteristics such as standard recoveries, number of common and specific compounds detected with suspect screening, number of common and specific unknown features detected, and range of molecular weight or polarity. For bioassays, the evaluations were assessed on the response of four nuclear receptors (estrogenic, androgenic, pregnane X, and aryl hydrocarbon receptors).

Based on the outcome of the results obtained for these tests, a single extraction protocol offering the best efficiency compromise for both chemical analysis and in vitro bioassays will be presented.

3.01.P-Tu206 Development of an Analytical Method for Dissociative Acidic Herbicides in Agricultural Commodities for Food Safety Monitoring

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Pesticides are essential for enhancing agricultural productivity and quality, with each country setting maximum residue limits (MRLs) suited to its agricultural conditions. Although global MRLs have been established for roughly 1,200 pesticides, 655 remain without MRLs in Korea, highlighting the need for analytical methods to monitor these compounds in imported foods. This study prioritizes dissociative acidic herbicides, including acifluorfen, bromoxynil, cloransulam-methyl, cyclanilide, ioxynil, picloram, propoxycarbazone (and its metabolite 2-hydroxypropoxycarbazone), pyrasulfotole (and its metabolite desmethylsulfotole), and thienencarbazone-methyl, for food safety management. These herbicides were previously incompatible with QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe)-based multi-residue methods due to their dissociative properties and high-water solubility. A modified QuEChERS-based analytical protocol was thus developed and optimized for five representative agricultural commodities (brown rice, soybean, potato, orange, and pepper). The workflow covering extraction, partitioning, purification, and determination was tailored for regulatory use. LC-MS conditions were optimized, including adjustments to column type, mobile phase composition, and other instrumental parameters. Given the herbicides' dissociative and acidic nature, extraction was enhanced with 1N HCl acidification followed by acetonitrile extraction. Magnesium sulfate levels were optimized to avoid

reverse partitioning into the water phase, and a buffer (EN method) stabilized the pH of agricultural extracts. Purification was refined by testing various dispersive solid-phase extraction (d-SPE) materials, including alumina, C18, PSA, PRiME HLB, and Z-sep+, with the most effective combination selected based on extraction efficiency and minimal matrix effects across the five commodities. The residue analysis method optimized in the previous steps was fully validated in terms of selectivity (no interferences), matrix effect ($|\text{ME}| \leq 50\%$), limit of quantitation ($\leq 0.01 \text{ mg/kg}$), linearities ($R^2 \geq 0.98$), accuracy (70~120%), and precision ($\leq 20\%$ RSD) according to the criteria of the European SANTE/12682/2019 guidelines. The validated method was then applied to monitor imported agricultural products, demonstrating its suitability as a regulatory tool for compliance and food safety management.

3.01.P-Tu207 The Molecular Structure and Composition of Indoor Dust Revealed by Comprehensive Multiphase Nuclear Magnetic Resonance Spectroscopy: Implications for Indoor Chemical Exposure and Risk Assessment

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A significant portion of human chemical exposure occurs indoors, but large variability between indoor spaces makes risk assessment and source apportionment difficult, limiting regulatory action and public health recommendations. Indoor dust is an important reservoir of and vehicle for exposure to semivolatile chemical contaminants such as plastic additives, flame retardants, pesticides, and personal care products additives in indoor environments. As such, dust is widely studied to identify new chemical contaminants and to monitor concentrations of legacy contaminants indoors. However, dust is a highly heterogeneous mixture of macroscopic particles, sorbed organics, metals, and other components, the molecular structure of which may be highly variable depending on the location sampled. Understanding this structure and variability is important to interpreting the role of dust in the indoor environment, as it will alter how indoor contaminants partition or sorb to dust and the availability of these contaminants for indoor chemistry or human exposure.

We applied comprehensive multiphase nuclear magnetic resonance spectroscopy (CMP-NMR) to ten distinct dust samples sourced from various locations from two residences in Toronto, Canada, a standard reference dust, and several other reference materials expected to be present in indoor dust samples. CMP-NMR allows chemical characterization of solid, liquid, gel-like, and semisolid components within a material, and when coupled with 2-dimensional NMR can provide a comprehensive picture of the molecular structure and composition of a material. Our results show remarkable similarities between the solid and gel phases of the indoor dusts across the three locations due to the universal presence of humans and fabric materials. However, there are distinctions between the soluble or mobile components, particularly between dusts sourced from different locations and between dust vacuumed off floors and dust gathered from air purifiers or settled on surfaces. These mobile and semi-mobile components contain information about dust age, source, and how indoor contaminants interact with the dust samples. Our findings provide insight into the properties and composition of indoor dust, an important vehicle for indoor chemical exposure and a key matrix for assessing chemical concentrations in indoor environments and will inform indoor chemical fate models and indoor exposure and risk assessment calculations.

3.01.P-Tu208 Increased (S)VOC Identification in Complex Samples using a Dual-Ionization EI&CI-TOFMS Hyphenated with a Flow Modulated GCxGC System

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Hyphenation to GCxGC sets high requirements to a mass spectrometric detector. Narrow chromatographic peak widths require high mass spectral acquisition rates that are commonly achievable by EI-TOF instrumentation. Flow modulation GCxGC simplifies the experimental setup but also increases the demands to the MS due to enlarged column flows, resulting in high split ratios preceding the detector. However, when measuring complex samples or unknown compounds an additional soft ionization technique, such as CI improves the compound identification process. For one dimensional GC the combination of EI and CI operating simultaneously on one analyzer within one single chromatographic run has been presented previously. In this study we show the compatibility and advantages of a fast-switching EI&CI-TOFMS coupled to flow modulation GCxGC.

Ambient air and a standard gas mixture were collected on desorption-tubes. The collected samples were desorbed, preconcentrated and injected into the GCxGC system including a flow-modulation setup. A standard GCxGC-method was provided by the collaborator. The EI&CI-TOFMS operating CI- and EI was

used as detector to provide simultaneous molecular (CI) and structural (EI) information. The hyphenation of a dual ionization EI&CI-TOFMS with a flow modulated GCxGC setup is presented. Special focus is set on: (1) characterization of the alignment of simultaneously generated EI and CI information; (2) the EI/CI switching behavior of the MS; (3) the chromatographic performance of the ionization sources; and (4) the advantages of using flow modulation GCxGC in combination with medium pressure CI sources. The complexity of the GCxGC-dual ionization MS coupling is addressed and experiments with the aim to harness the high gas flows used in flow modulation GCxGC were conducted. It can be shown that the high pumping efficiency in the CI interface can be utilized to increase the CI sensitivity by a factor of 30, by guiding the larger share of the flow ratio into the CI source. Using a specifically defined flow splitting, it was possible to maintain the flow balance within the EI pumping stage and simultaneously increase the CI performance leading to increased identification certainties as well as decreased limits of detection (LOD). The findings on the instrumental performance investigated via standard gas mixture measurements were further verified on ambient air samples collected at a federal highway in Germany.

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3.01.P-Tu209 Strategies and Approaches for Particulate Matter Analysis in Air

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Particulate matter (PM) in ambient air is a major environmental concern due to its impact on human health and the environment. PM levels fluctuate due to various factors, including industrial activity, vehicle emissions, and weather conditions.

Accurate monitoring and analysis of particulate matter, particularly in urban environments, remains a challenge due to the complexity of sources and the variability in PM composition.

This study explores the application of GED-SP-ICP-MS (Gas Exchange device - Single Particle - Inductively Coupled Plasma Mass Spectrometry) for the detection and analysis of particles in gaseous media. We aim to assess the viability of this technique for monitoring both environmental air samples and gas samples from industrial sources.

This poster presentation will demonstrate the potential of GED-SP-ICP-MS to improve particulate matter monitoring in air quality studies, with direct applications in public health, environmental monitoring, and industrial hygiene. This technique could lead to more accurate assessments of pollution levels and sources, guiding regulatory efforts to reduce exposure to harmful particulate matter.

3.01.P-Tu210 Identification of Urinary Biomarkers and Biotransformation Pathways of DEHCH: A Step Toward Improved Biomonitoring of Non-Phthalate Plasticizers

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Non-phthalate plasticizers are increasingly used as safer alternatives to phthalates due to concerns about human toxicity. Among these, bis(2-ethylhexyl)cyclohexane-1,4-dicarboxylate (DEHCH) is a newer option. This study aimed to investigate the biotransformation of DEHCH in humans to identify phase I metabolites and develop biomarkers for exposure assessment. Using human liver microsomes in vitro and urinary samples collected after a single oral dose, primary and secondary metabolites were characterized. Cyclohexane-1,4-dicarboxylate-mono-2-ethyl-hexyl ester (MEHCH) was identified as the primary metabolite, formed through hydrolysis of DEHCH. Secondary oxidized metabolites included cx-MEHCH, OH-MEHCH, and oxo-MEHCH, while complete breakdown products such as cyclohexane-1,4-dicarboxylate (CHDA) were also detected. Quantitative analysis revealed that over 48 hours, urinary excretion mass fractions were 0.4% for MEHCH, 3.4% for OH-MEHCH, 0.3% for oxo-MEHCH, 0.2% for cx-MEHCH, and 51.3% for CHDA. This is the first study to identify a urinary biomarker for DEHCH exposure in humans, laying the groundwork for future biomonitoring research on non-phthalate plasticizers.

3.01.P-Tu211 Enhanced Nanoplastics Detection via Optical-Photothermal Infrared (O-PTIR) Coupled with Simultaneous Raman Spectroscopy

Miriam Unger¹, Natalia P. Ivleva², Carolin Borbeck¹ and Marcel Klotz², (1)Photothermal Spectroscopy

Introduction

The global prevalence of plastics has led to widespread environmental contamination with microplastics (1 μm to 1 mm) and nanoplastics (<1 μm). Nanoplastics are especially hazardous due to their ability to penetrate biological barriers and carry toxins.

Method

Traditional detection methods, such as μ -Raman spectroscopy, provide high spatial resolution but are limited by fluorescence interference, while FTIR techniques lack sufficient spatial resolution. Optical-Photothermal Infrared Spectroscopy (O-PTIR) combines the spatial precision of Raman (532 nm) with IR's chemical specificity, enabling submicron IR resolution. It allows simultaneous collection of IR and Raman spectra, offering cross-validation and reducing interference.

Results/Discussion

This study presents a powerful method for identifying diverse classes of nanoplastics, with particle sizes down to 300 nm. By leveraging O-PTIR's dual-spectroscopy capabilities, simultaneous infrared and Raman data were collected, providing detailed chemical insights. This approach enhances the reliability of nanoplastic analysis by uncovering critical information about particle coatings and embedded dyes.

Conclusions

O-PTIR with simultaneous Raman provides a powerful approach for detecting and characterizing nanoplastics, overcoming limitations of traditional methods. This technique shows promise for environmental monitoring and health studies, supporting improved risk assessments of nanoplastic pollution.

3.01.P-Tu212 Determination of Food Microplastics using Depolymerization and Ultra-Performance Liquid Chromatography/Tandem Mass Spectrometry

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Plastics can degrade into microplastics smaller than 5 mm, potentially entering humans via the food chain and causing adverse health effects. Current microplastic detection methods, such as Fourier-transform infrared (FTIR) and Raman spectroscopy, are prone to false positives and cannot detect nanoparticles; also, results may vary due to differences in cut-off particle sizes. Depolymerization followed by monomer analysis offers a potential solution, but existing studies mainly focus on environmental samples. This research thus aims to develop a depolymerization method to detect microplastics of polyethylene terephthalate (PET), polycarbonate (PC), polylactic acid (PLA), Polyamide 6 (PA 6), and Polyamide 66 (PA 66) in food matrices.

This investigation employs conventional reflux and microwave-assisted acid digestion methodologies for plastic depolymerization. PET, PC, and PLA microplastics were treated with 1.0 g of KOH in 20 mL of 1-pentanol at 135°C for 45 minutes using a reflux condenser. The resultant decomposed monomers of PET, PC, and PLA are terephthalic acid (TPA), bisphenol A (BPA), and lactic acid (LA), respectively. PA 6 and PA 66 underwent a reaction with 2 mL of 20% hydrochloric acid (HCl)(aq) using CEM MARS for microwave-assisted depolymerization at 170°C for 45 minutes, yielding 6-aminocaproic acid (6-ACA) and adipic acid (AA) monomers, respectively.

The monomer concentrations were determined utilizing Waters ultra-performance liquid chromatography-MS/MS (UPLC-MS/MS) with UniSpray ionization. TPA, AA, and LA were separated on a Waters ACQUITY UPLC BEH Amide column, whereas BPA and 6-ACA were separated on a Supelco Ascentis Express F5 column.

The basic depolymerization efficiencies of PET, PC, and PLA attained 99%, 116%, and 180%, respectively, demonstrating that this procedure could fully depolymerize the three plastics. The elevated efficiency of PLA resulted from the presence of LA from reagents. For PA6 and PA66, the acid microwave-assisted depolymerization achieved 91% and 85% efficiencies, respectively.

This investigation presented a methodology for quantifying the concentrations of five categories of microplastics, yielding comparable results. The obtained concentration measurements were valuable for exposure assessment and provided empirical evidence to inform the development of regulations to mitigate microplastic pollution.

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3.01.P-Tu213 Enhanced Digital Quantification of Microplastics using Sequential Nile Red and Rose Bengal Staining

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Despite microplastics (MPs) being an increasingly prominent global concern, there are currently no standardized methods for their visual analysis. This is a crucial step towards understanding the extent of pollution and its environmental impacts. In the context of the project LIFE CASCADE, the authors explored an innovative visual analysis technique utilizing a digital microscope instead of a conventional optical microscope. To enhance optical quantification, they employed the Keyence Series VHX-7000 digital microscope, paired with the Keyence VHX-7100 camera and VHX-E100 lens, which offers a remarkable resolution of up to 2 µm. This setup simplifies the basic operations necessary for quantifying MPs, making it user-friendly and accessible, even for beginners. The high-resolution images produced are comparable to those obtained from scanning electron microscopy (SEM).

Furthermore, the quantification process was refined through the sequential use of two of the most commonly utilized dyes in microplastics analysis: Nile Red and Rose Bengal. Nile Red is a well-established dye for MP quantification, providing a straightforward method to reduce the number of particles that require confirmation through analytical techniques such as Fourier-transform infrared (FTIR) or Raman spectroscopy. However, its affinity for biogenic materials can sometimes result in false positives, leading to an overall overestimation of particle counts. In contrast, Rose Bengal exhibits a stronger affinity for natural organic matter, which helps to mitigate the errors associated with Nile Red. By integrating the digital microscope and employing a sequential combination of these two dyes, the authors aimed to present an enhanced approach to visual quantification of microplastics. This method facilitates the rapid differentiation between plastic particles and natural organic materials after digestion, providing a fast and more reliable tool for counting microplastic fragments in environmental water samples.

3.01.P-Tu214 Comprehensive Pesticide Screening with Dual Ionization GC-HRMS: Bridging Targeted and Non-Targeted Analysis

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The detection and quantification of pesticide residues and contaminants in agricultural products are critical for ensuring food safety and compliance with global regulations. The growing demand for these requirements, driven by consumer awareness and stringent regulations, increases the demands on analytical screening. Chromatographic techniques coupled to high-resolution mass spectrometry (HRMS) are becoming a most reliable analytical platforms for pesticides analysis, mainly due to their comprehensive detection, and identification of both, known and unknown residues with high sensitivity and specificity. Here, we present a new non-targeted analysis approach of volatile pesticide residues in food matrices. For unambiguous compound identification a novel gas chromatography (GC)-HRMS system generating simultaneously EI and CI information on the detected analytes was utilized.

Agricultural products were extracted using QuEChERS protocols and analyzed with a GC 8500 system (Bruker, Germany) coupled to an ecTOF detector (Bruker, Germany). The system's dual ionization capability, combining electron ionization (EI) and chemical ionization (CI) simultaneously, provides complementary structural and molecular data. CI reagent ions (N₂H⁺, H₃O⁺, NH₄⁺) were used to adjust fragmentation and enhance molecular detection. A targeted library of 213 pesticides was created for targeted analysis. Data post-processing for targeted, suspect and non-targeted approaches were carried out with AnalyzerPro XD (Spectralworks, UK).

The GC-HRMS system effectively identified pesticide residues with certainties above 70% for both EI and CI traces using the created targeted library. Non-targeted analysis detected additional compounds that were analyzed using NIST library searches and accurate mass data. Added CI information improved sensitivity and enabled residue detection at low ppb levels. The dual-ionization approach enhanced compound identification certainties, reduced analysis time, and increased reliability for complex samples. The integration of EI and CI in a single GC-HRMS workflow enhances certainties and reliability for both targeted and non-targeted pesticide residue analysis. The system shows to be a highly efficient tool for ensuring food safety and regulatory compliance, offering robust solutions for identifying pesticides in complex agricultural products.

3.02.A Advances in Exposure Modelling Toward a Safe and Sustainable Tomorrow for Both Humans and the Environment

3.02.A.T-01 Exposure Modelling in Europe: How to Pave the Road for the Future as Part of the European Exposure Science Strategy 2020–2030

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Exposure modelling is essential in almost all relevant contexts of exposure science. To address the numerous challenges that exist, exposure modelling is one of the priority areas of Europe Chapter of the International Society of Exposure Science (ISES Europe). Therefore, a strategy was developed, focussing on this priority area, with four strategic objectives:

- (1) improvement of existing models and modelling tools,
- (2) development of new methodologies and support for understudied fields,
- (3) improvement of the use of models and training for model users, and
- (4) development of models to fulfil regulatory needs and improve acceptance

Exposure modellers from a wide range of European countries and institutions, active in the fields of occupational, general population (humans via the environment and exposure due to handling chemicals by consumers) and environmental exposure science pooled their expertise under the umbrella of the ISES Europe working group on exposure models. This working group assessed the state-of-the-art of exposure modelling in Europe. They developed an inventory of exposure models used in Europe and reviewed the existing literature on pitfalls associated with exposure modelling, in order to identify crucial modelling-related strategy elements. Additionally, an online survey was conducted and disseminated to the European exposure science community to identify modelling training needs and a repository of guidance and standard for regulatory exposure modelling was developed.

Decisive actions were defined for ISES Europe stakeholders focused on achieving the long-term goal of developing a best practice in exposure modelling. Additionally, to the four strategic objectives, the working group developed an action plan and roadmap for the implementation of the European Exposure Science Strategy for exposure modelling. This strategic plan shall foster a common understanding of modelling-related methodology, terminology, training and education, as well as future research in Europe, and have a broader impact on strategic considerations globally.

3.02.A.T-02 Identification of Environmental Release Hotspots Along the Product Life Cycle Through HotSpot Scan Tool

Neeraj Shandilya, Lia de Simon, Tom Ligthart, Eugene van Someren and Wouter Fransman, TNO, Netherlands

Early identification and quantification of potential harmful emissions to environmental compartments (including air, water and soil) throughout a product's life cycle are crucial for a safe and sustainable by design approach. The *in silico* HotSpot Scan tool facilitates this by offering a quick, and low-input assessment, using an extensive data inventory to pinpoint life cycle stages and environmental compartments with potential emissions. These are identified as hotspots or focal points during the life cycle of a product.

Its applicability was demonstrated on the development of perovskite-based solar cell devices which show high solar power conversion efficiency with low production costs (i.e., highly interesting from cost v/s performance perspective), but suffer from poor operational and structural stability and the toxicity of the used materials (i.e., major concerns for safety and sustainability), which can hinder their practical commercialization and widespread application.

The results show that approx. 80% of the total emissions to the environment occur during the end-of-life phase of the device, mainly affecting ground water. Thus, a viable recycling method is critical for improving the device safety and sustainability. Lead emissions were estimated throughout the device life cycle. Several toxic substances used during the device production were also estimated to be emitted (approx. 10% of the total emission), mostly to the wastewater. This includes lead nitrate, lead halide, methylammonium halide, dimethylformamide and precursor ink. Substituting these chemicals with less hazardous ones is thus needed. The released amounts to (particularly indoor) air were estimated to be relatively lower due to the risk management measures put in place during synthesis and manufacturing of the perovskites, and even lower to the soil. The remaining emissions were linked to the device use and breach of the perovskite encapsulation layer which can lead to leaching of the lead ions into the soil. Thus, structural stability of the encapsulation layer is critical.

This case study demonstrates that HSS is a valuable tool for quickly assessing mass flows and environmental releases throughout a product's life cycle. The publicly available online tool allows

innovators to proactively adjust designs, minimizing releases and supporting safer and more sustainable products.

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3.02.A.T-03 Predicting the Fate of 40 Emerging Contaminants: A Case Study of SimpleTreat and TOXCHEM

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Modelling micropollutant fate during wastewater treatment can help practitioners improve treatment conditions and maximise micropollutant removal. We evaluated two fate models, SimpleTreat and TOXCHEM, for predicting removal efficiencies of 40 micropollutants (19 pharmaceuticals, 7 PFAS, 6 flame retardants (BDEs), 2 heavy metals, 2 PAHs, 1 pesticide, 1 antifungal, 1 corrosion inhibitor, and 1 plasticiser). Predicted values were compared with observed values obtained from monitoring studies of 36 sewage treatment plants across the United Kingdom as part of the National Chemicals Investigations Program (CIP) conducted from 2011-2021. Predicted removals exhibited average variance from observed removals at 28.5% for SimpleTreat and 37.4% for TOXCHEM. The results indicate that SimpleTreat is more fit for purpose. However, both models exhibit discrepancies between simulated and observed values. For micropollutants, where practitioners have a better understanding of their chemical properties, i.e., pharmaceuticals, both models had better predictions closer to reality with deviations of less than 30% (e.g., Erythromycin observed value 18% vs predicted TOXCHEM value at 15%). However, for BDEs and PFAS, the error reached up to 100% (e.g., PFHxA estimated 91.6% (SimpleTreat) or 22.5% (TOXCHEM) compared to the observed negative removal at -42%). In both fate models, it was observed that the predictions were more sensitive to a compound's input chemical properties rather than the treatment plant's input operating conditions (flow rate, BOD, suspended solids). That verifies that the derived discrepancies are due to the models physicochemical properties used to predict micropollutant fate. For instance, in the case of PFAS, micropollutants with complex structures, it is difficult to obtain accurate information about their chemical properties in the environment. Improving accuracy requires a better understanding of micropollutants' behaviour in real environmental conditions. Another way to improve predictions is to incorporate data-driven models (machine learning) into current fate models. Data-driven approaches make predictions based on measured values and do not rely on chemical properties minimising the errors derived from input chemical properties.

3.02.A.T-04 A Framework for Projection of Future Chemical Emissions Under the UK Shared Socioeconomic Pathways

Hongyan Chen, Jacky Chaplow, Sam Harrison and STEPHEN LOFTS, UKCEH, United Kingdom

Chemical emissions into the environment pose risks to human health and ecosystems. Understanding these emissions and exploring their evolution under the uncertainties of future climate and socioeconomic changes is critical for effective long-term management. However, few studies have focused on this issue, particularly at finer spatial scales. This study introduces a novel framework to address these challenges, integrating use profiles of chemicals to identify emission proxies based on release pathways, a scenario analysis toolkit to estimate proxy trends under selected representative concentration pathways (RCPs) and shared socioeconomic pathways (SSPs), and a downscaling approach to spatialise trends and emissions at a 5 km grid scale in the UK. The scenario analysis toolkit, a key component, enables users to select proxy drivers from 50 semi-quantitative variables in UK_SSPs, define their effects, including their direction and weight, and automatically generates proxy trends through weighted sums. This process is transparent, efficient, and repeatable, fostering stakeholder engagement, which is crucial for informed decision-making. The framework is demonstrated through a case study analysing Fipronil emissions to freshwaters from flea treatments for household pets in the UK. The resulting spatiotemporal emission data provide a foundation for further modelling of chemical fate and impacts, supporting the prediction and management of chemical risks.

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3.02.A.T-05 Towards the Assessment of Risk of Contaminants of Emerging Concern in Surface Waters using HydroFATE, a Global Contaminant Fate Model

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Contaminants of emerging concern in surface waters pose significant risks to both the environment and human health. While comprehensive monitoring of such contaminants in surface waters is essential, resource and analytical constraints often hinder the feasibility of widespread assessments, thereby necessitating the use of contaminant fate models as a basis for risk assessment. Existing models generally require specialized technical expertise and substantial computing power, limiting their accessibility for stakeholders involved in water management. To address these challenges, we introduce HydroFATE, a global contaminant fate model designed as an accessible tool for screening risks associated with contamination of surface waters due to chemicals associated with human consumption. HydroFATE enables stakeholders including chemical and pharmaceutical industries, wastewater treatment facility operators, regulatory agencies, policymakers, and environmental consultants to efficiently prioritize monitoring and regulatory compliance efforts. Although the development of a more user-friendly interface is currently underway, HydroFATE's current framework already allows for scientifically robust and rapid assessments, making it a valuable resource for informed decision-making. Initial applications of the model have demonstrated its effectiveness in predicting antibiotic pollution in surface waters worldwide. Ongoing efforts to expand HydroFATE to model a broader range of contaminants will further enhance its utility across the globe. By fostering collaboration with government agencies, non-profit organizations, and industry stakeholders, we aim to encourage the widespread adoption of HydroFATE. This initiative will support evidence-based policy development, ultimately contributing to the preservation and improvement of the global water quality and ecosystem health.

3.02.B Advances in Exposure Modelling Towards a Safe and Sustainable Tomorrow for Both Humans and the Environment

3.02.B.T-01 An Integrated Water and Air Modelling Study for a Comprehensive Evaluation of Shipping Environmental Impacts in the Northern Adriatic Sea

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Marine transport is a cornerstone of the global economy, carrying around 90% of world trade by volume and facilitating the movement of people, essential goods, raw materials, and energy resources across continents. However, marine water quality and coastal air quality degradation due to shipping activities have been recognized as a significant concern and related to negative effects on both the environment and human health.

The aim of this study was to investigate using high-resolution modelling tools the impact of shipping on air and water quality in the Northern Adriatic Sea with respect to other land-based sources. In detail two metals (i.e., cadmium Cd and lead Pb), two polycyclic aromatic hydrocarbons (i.e., benzo-a-pyrene BaP, and fluoranthene Fl), and four air pollutants (i.e., ozone O₃, sulphur dioxide SO₂, nitrogen oxides NO_x, and particulate matter PM_{2.5}) were selected due to data availability for model development and validation. The Ship Traffic Emission Assessment Model (STEAM) model was used to estimate the discharge of liquid wastes and the emission to the atmosphere of air pollutants from shipping activities, while data on other sources was obtained from monitoring data and international emission inventories. Water quality modelling was carried out by applying the ChemicalDrift module of the OpenDrift suite using forcing data from the Shallow water HYdrodynamic Finite Element Model (SHYFEM) model and Copernicus Marine Services. The System for Integrated modelLling of Atmospheric composition (SILAM) model used to track shipping emissions into the atmosphere using meteorology data from the European Centre for Medium-Range Weather Forecasts (ECMWF) and from the Weather Research Forecasting (WRF) model.

The applied modelling framework allowed to comprehensively evaluate the combined impacts of shipping to water and air quality in the Northern Adriatic Sea. Shipping traffic was shown to contribute significantly to air quality, especially to SO₂, NO_x, and PM_{2.5} concentrations, as well as to NO_x, Cd, and Pb deposition in the open sea. Shipping contribution to water pollution was shown to be significant especially along shipping lanes, while the contribution to sediments quality was more limited. While shipping has been identified as a significant source of several pollutants, further studies should also consider the inherent ecotoxicological effects of all shipping effluents (e.g., scrubber water, bilge water, and ballast water).

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3.02.B.T-02 Reconciling Plastic Release: Comprehensive Modeling of Macro- and Microplastic Flows to the Environment

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The presence of plastic all over the world is one of the major environmental issues of the 21st century. In order to tackle this problem, knowledge about environmental emissions is crucial. Material flow analysis combined with release assessments is one way to quantify plastic emissions from a whole product life cycle perspective. This study integrates recent research on key emission pathways into a plastic release model based on probabilistic material flow analysis, and comprehensively models macro- and microplastic emissions of seven polymers in Switzerland for the year 2022. Our model estimates that 222 ± 50 g/capita/year plastics are released into the environment, with PET contributing 37%, followed by PP of 22%, PVC 13%, HDPE 12%, LDPE 8%, EPS 4%, and PS 3%. Fiber release from textiles and pellet loss during pre- and post-consumer processes were identified to be primary microplastic release pathways. For macroplastics, the main release processes are littering of packaging products and losses during post-consumer collection. Soil is identified as the primary receiving compartment for plastic emissions, accounting for 98% of macroplastics and 94% of microplastics, respectively. A comparison with previous release estimates shows a large variability of per-capita emissions, mainly caused by inclusion or not of rubber release from tyres and differences in modeling the littering and dumping processes.

3.02.B.T-03 PROTEX: Chemical Safety and Sustainability Assessment from Production Lines to Human and Ecological Receptors

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Understanding human and ecological exposures to human-made chemical substances necessitates holistic and systematic information about chemical emissions from multiple lifecycle sources, fate in multimedia environments at multiple scales, multiple sources of exposure, and multiple pathways of intake and elimination. These components are governed by the complicated interactions between chemical properties, environmental conditions, and human and ecological characteristics. Such multidimensionality and complexity warrant a systematic, mechanistic perspective in exposure modeling and necessitate a holistic approach to track a chemical's journey from the production line to the body burden. In this presentation, we introduce a state-of-the-science model named PROduction-To-EXposure (PROTEX), which supports characterizing the entire continuum from chemical production, lifecycle multi-source emissions, multi-scale multimedia environmental fate and transport, and multi-pathway exposure, to the presence in humans and other organisms. PROTEX builds on theories of substance flow analysis, multimedia fate and transport, bioaccumulation, and exposure and toxicokinetics, and it integrates mechanistic descriptions of various physical, chemical, biological, and socioeconomic processes. Its mechanistic nature allows PROTEX to be parameterized for a wide range of chemical substances (both neutral and ionizable chemicals) in a wide range of regional environments (e.g., the subtropical U.S., the Canadian side of the Lake Ontario basin, central China, and the western Baltic drainage basin) for a wide range of populations (Whites, Blacks, Asians, etc.). When supplied with time-variant production or use data, PROTEX provides dynamic time-dependent estimates, allowing users to reconstruct the long-term history of chemical contamination. By considering interindividual variabilities in human anthropometrics, behavior, and physiology, PROTEX additionally supports predicting the distribution of chemical exposure within a population. Outputs from PROTEX support science-based risk assessment and decision-making. The presentation serves as a knowledge hub for PROTEX's rationale and mechanism, applications and case studies, and user tips.

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3.02.B.T-04 Exploring the Long-term Human Exposure to Short-, Medium-, and Long-chain Chlorinated Paraffins Under Variant Environmental Release Trends and Patterns

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Chlorinated paraffins (CPs) have been extensively released into the environment, resulting in pervasive and long-term human exposure on a global scale. Patterns of CP use and release vary significantly across regions, differing by chain length (short-, medium-, or long-chain CPs), usage context (indoor vs. outdoor), and temporal trends. In this presentation, we apply a modeling approach to investigate how these regional differences influence the extent and pathways of long-term CP exposure in human populations. Focusing on three case regions China, Canada, and Northern Europe we specifically examine: (1) the relative contributions of far-field versus near-field exposure across regions, driven by differing CP use and release patterns; (2) variations in temporal trends of human body burdens within longitudinal cohorts, identifying which generations experience the highest exposure levels; and (3) whether our modeling approach can explain the cross-sectional body burden trends with age observed during biomonitoring studies. Release trends for SCCPs, MCCPs, and LCCPs that vary across the three regions translate to highly divergent longitudinal concentration age trends. For example, Chinese cohorts born after the 1990s experience rapid whole-body concentration increases, whereas European cohorts show declining concentrations over their lifetimes. Predicted peak body concentrations occur at different times in different regions and or different CP groups. Regional differences in CP use contexts do not appear to significantly alter the relative importance of far-field versus near-field exposure pathways. By identifying key exposure drivers and vulnerable populations, this work supports the efficient management of CP-related health risks and offers a valuable framework for evaluating the effectiveness of future CP emission reduction strategies.

3.02.B.T-05 What Does the Next Generation of Environmental Exposure Models Look Like?

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Environmental models and software are essential tools for understanding the complex interactions of the natural world. They empower us to foresee potential futures, unravel intricate trends and expand our scientific knowledge, ensuring we make informed decisions for a sustainable future. This includes environmental emissions and exposure models, which tell us how chemicals and other potential pollutants enter, move around and behave in the environment. To achieve accurate, efficient, collaborative and integrative insights into this pollution and its sources, it is imperative that our models and software keep pace with scientific and technological advances, the increasing availability of data and a heightened importance on assessing complex, interconnected systems under a changing climate. But often our models follow outmoded programming paradigms and technological setups that makes this difficult. They are often monolithic codebases rather than flexible, standalone modules, making them difficult to adapt to emerging risks or integrate with other models to predict, e.g., the societal drivers and One Health impacts of pollution. Furthermore, modelling efforts often overlook ethical and sustainability issues, like the carbon footprint of running complex simulations or the societal impacts of using model predictions to inform policy. These considerations framed a workshop that took place in October 2024, bringing together interdisciplinary environmental modellers from around the world to discuss the question: what does the next generation of environmental models look like?

The focus was interactive sessions, where participants discussed this question by referererring to six pillars:

Software engineering and collaborative platforms; Interdisciplinary learning; Cloud-based and exascale computing; Citizen science; Artificial intelligence, and; Big data and better monitoring. In this presentation, we reflect on the outcomes, placing them in the context of emissions and exposure modelling.

The workshop was part of broader efforts to build an international community of practice around environmental modelling. A priority identified is that training, education and knowledge transfer are vital to ensuring that we empower the next generation of environmental modellers, as well as the models themselves, and we hope this community will provide a space to enable this.

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3.02.P Advances in Exposure Modelling Toward a Safe and Sustainable Tomorrow for Both Humans and the Environment

3.02.P-We172 Overall Persistence as a Time-Dependent Parameter

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Chemical persistence plays a key role in the determination of environmental exposure making it an important endpoint in risk assessment and regulation. For persistence assessment information is required on the degradation rates in different compartments (soil, water, sediment, air). However, degradation rates are prone to wide variability depending on environmental conditions, and evaluating degradation half-lives using a compartment-by-compartment approach is overly simplistic, and neglects dynamic multimedia exchanges and degradation processes. According to the REACH guidance R.11, results from multi-media modelling could be used case-by-case in order to evaluate the environmental exposure and compartment(s) of specific concern. Overall persistence (Pov) has been proposed to be a suitable replacement metric for the compartment-specific half-lives in persistence assessment. However, overall persistence at steady state depends heavily on the release pattern, whereas the temporal remote state, defined as the state of the model system long after the stop of emissions, is independent of the release pattern of a chemical.

In this presentation, influencing parameters of overall persistence over time will be illustrated. The multimedia model MUST (level III and IV) is applied on case studies taking into account different properties (degradation half-lives, volatility, solubility, adsorption), and different mode of entries. Sensitivity analysis will support evaluation of most important input parameters. In addition, emission scenarios such as emission stop are used to evaluate the impact on the overall persistence and the relevant compartment.

Overall persistence is given usually for the steady-state concentration, and will usually be given as residence time, which has to be converted into half-lives to be comparable with persistence triggers. However, overall persistence at steady-state depends on release pattern, Moreover concentration in the different compartments will change over time due to change in release pattern, distribution and different degradation rates. For this reason, overall persistence is a time-dependent parameter (Pov(t)) as well, which can be illustrated by Level IV simulations. Further influencing factors determined by sensitivity analysis will be presented.

3.02.P-We173 Enhancing Practice: The ISES Europe Repository of Good Modelling Practice (GMP)

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Background: The European Exposure Science Strategy 2020 2030 aims to enhance the use of computational exposure models by providing model users with essential resources, including standardized exposure scenarios, guidance on exposure modelling, regulatory guidelines, and efficiency factors for risk management measures.

To support these objectives, a dedicated subgroup was formed within the Exposure Models Working Group of the International Society of Exposure Science (ISES) Europe Chapter.

Methodology: The subgroup's first goal was to create a free resource that promotes Good Modelling Practices (GMP) in exposure science. As outlined by the subgroup, this involves identifying suitable model input parameters, selecting and using exposure models effectively, evaluating models and results

appropriately, and clearly interpreting and communicating outcomes to all stakeholders.

Results and Discussion: Over the past two years, the subgroup populated a Repository of Good Modelling Practice (GMP) with input from the ISES Europe community. It includes links to over 300 resources, such as guidance documents, scientific publications, databases, and webpages. The focus is on European references published after 2000. Launched in late 2024, the Repository is freely accessible on the ISES Europe website. It is organized into five key themes to support the modelling process: (1) Substance Parameters, (2) Exposure Factors, (3) Exposure Modelling, (4) Model Evaluation, and (5) Result Communication.

Future: As a living document, the Repository of Good Modelling Practice (GMP) will be updated periodically. Conference participants are encouraged to provide feedback and share additional references.

3.02.P-We174 From Complexity to Simplicity Toward Streamlining Safe and Sustainable by Design (SSbD) Implementation - The ECETOC Task Force's Approach to Practical Tools for Evaluating the Safety Dimension

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The European Union's Chemicals Strategy for Sustainability (CSS) aims to transition towards a safe and sustainable approach for chemicals. Central to this strategy is the Safe and Sustainable by Design (SSbD) concept, which promotes a holistic approach integrating safety, circularity, and functionality of chemicals, materials, products, and processes throughout their entire life cycle to minimize environmental footprints. SSbD is a rapidly evolving field requiring interdisciplinary collaboration and the integration of multiple perspectives and tools. Successful implementation depends on balancing regulatory requirements with business realities while maintaining a focus on meaningful safety and sustainability improvements. Despite the availability of numerous tools, challenges persist including identifying suitable tools and interpretations of assessments. The ECETOC Safe and Sustainability Task Force aims to evaluate existing tools from different perspectives including data requirements from supply chains, complexity of assessments, interpretation and comparison of outcomes, integration of safety and sustainability considerations, and clear boundaries in both safety and sustainability assessments through a series of case studies. These studies are designed to assess each tool's effectiveness and usefulness in real-world settings and to identify areas for refinement for safety dimensions. As a starting point, the task force has defined case studies using the ProScale tool as a Tier 1 assessment method for chemicals, mixtures, formulations, and articles to establish a methodology for comparing the safety of alternative product solutions and to evaluate the usefulness of the comparison outcomes. The proposed method focuses on formulations based on readily available safety information on ingredients, making it broadly applicable. This approach complements current methods, such as those tested by the Joint Research Centre (JRC), which focus on single chemicals and require extensive data and expertise.

This initiative seeks to bridge the gap between theoretical tool development and practical application. By inspiring and equipping stakeholders with the mindset, knowledge, and tools needed to drive meaningful change, it aims to ensure a safer and more sustainable future for both, humans and the environment. Additionally, it provides new ideas for testing of the advancements in practical case studies, deriving lessons learned, and showcasing success stories.

3.02.P-We175 Finding Practical Answers to Environmental Exposure Assessment Questions

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"ECETOC's Targeted Risk Assessment (TRA) tool is a widely accepted screening tool to assess chemical exposure risks for workers, consumers, and the environment in a regulatory context (e.g., under the REACH Regulation).

ECETOC assigns high priority to maintaining the TRA tool as a trusted resource in chemical safety assessments and to advancing the science of exposure assessment as a whole. To drive progress in this

field, ECETOC has established three expert Task Forces focused on identifying and addressing key challenges in exposure assessment for workers, consumers and the environment using the latest science. Exemplifying ECETOC's versatility in working with partners, the TRA Environment Task Force has recently investigated the following two significant questions using diverse collaborative frameworks.

3.02.P-We176 SimpleBox in R: A Multimedia Fate Model for Molecules, Engineered Nanoparticles and Microplastics

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SimpleBox is a screening level multimedia fate model for chemical substances (molecules, engineered nanoparticles and microplastics) that supports (European) chemical regulation (e.g., REACH) and Life Cycle Impact Assessment modelling (e.g., ReCiPe). The model comprises of boxes, which represent environmental compartments at different geographical scales. The original versions (1993, 1996, 2004, 2016) are implemented in Excel, which allows easy adaptation for specific use, for instance a version for nanomaterials or microplastics. But such implementations have several disadvantages, most importantly version control of both the data and the model and limiting the use to calculating deterministic steady-state masses and concentrations. We present a novel implementation of SimpleBox in R that overcomes the mentioned disadvantages and has other advantages as a bonus.

The design of the R version is object-oriented enabling a single codebase for general and specific use, and easy integration with R-shiny as user-interface for users not specialized in programming. Furthermore, SimpleBox in R has the advantage that the fate process algorithms are transparent and easily adapted for the intended scenario. This supports further model development.

The main features of SimpleBox in R: the ability to estimate masses in compartments steady-state and dynamically, both deterministically and probabilistically. Concentrations are quickly calculated using the included concentration module. SimpleBox is easily coupled with emission models, such as material flow analysis (MFA) models, both dynamically and probabilistically. The model uses one code base for chemicals, nanomaterials and microplastics, containing functions specific to the substance types.

SimpleBox in R is available via <https://github.com/rivm-syso/SBooScripts> and <https://github.com/rivm-syso/SBoo>

3.02.P-We177 The Evolving Full Multi: Flexibly Simulating Microplastic Exposure in Aquatic Environments

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Plastic pollution is pervasive in rivers, lakes and the global ocean, and threatens environmental and human health. The high complexity and heterogeneity of plastic pollution means it is challenging to monitor in the field, and understanding of source-to-concentration relationships is lacking. Computational models are essential for understanding the emission, transport and transformation of these novel entities in aquatic systems. The open-source Full Multi framework, first released in 2022, is a process-based mass-balance model that can comprehensively describe the behavior of microplastics with varying physical properties in multiple size ranges and multiple aggregation states in lakes, rivers or the ocean. First-order kinetics are used to describe the exchange of microplastic particles between model compartments. When applied to river systems, the model includes compartments representing flowing water at the surface, the flowing water column, stagnant water near the sediment surface, and sediments. In the original Full Multi model, the description of settling and mixing of plastic particles in rivers was simplistic - based on a generic parameterization of Stokes law - and failed to account for turbulent mixing. Here, we describe an improved description of mixing and resuspension in rivers in the Full Multi which relates vertical transport rates to river velocity. In the new version of the model, rates of microplastic deposition and erosion from bed sediment to the water column and burial in sediment are dependent on horizontal flow velocity of the river. Model results are consistent with expectations derived from the well-known Hjulström Curve and the dimensionless shear stress baseline. This suggests that the evolving Full Multi model will be able to dynamically simulate the fate and transport of microplastics in river systems, and ultimately, estimate microplastic transport rates from rivers to the oceans with better fidelity to the real environment.

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3.02.P-We178 Simulating Chronic Exposure to DEHP with a PBPK Model: First Steps Toward Aggregate Exposure with PK-Sim

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Aggregate exposure (AE) is an individual's combined exposure to a single chemical by different routes of exposure (topical, oral, and inhalation) from various pathways (air, water, food, soil, dust, and contact with surfaces) that occur at other times and locations, as the result of multiple sources of exposure.

AE varies across individuals, and over time, assessments of populations are described in terms of distributions of short-term doses (acute exposures) and long-term average doses (chronic exposures). Di-2-ethylhexyl phthalate (DEHP), an industrial chemical extensively used in polyvinyl chloride (PVC) plastics, presents a significant concern for AE assessment due to its widespread presence in consumer products such as shoes, gloves, packaging materials, building materials, pharmaceuticals, personal care products, food contact materials, paints, and adhesives.

In this study, we apply an open-source physiologically based kinetic (PBK) model, specifically PK-Sim, to predict the overall internal dietary and consumer AE of DEHP. PBK models are particularly relevant for AE assessments as they can account for exposure from various sources, routes, and timeframes, providing accurate estimations of exposure levels. Furthermore, PBK models can support the prediction of plasma and tissue levels across different populations and life stages, enabling quantitative estimates for individuals with higher exposure risks.

Our PBK model incorporates key parameters related to DEHP metabolism, including the synthesis of its hydrolysis product mono-2-Ethylhexyl phthalate (MEHP) and its elimination through renal excretion. By integrating available estimates of consumer and dietary exposure over prolonged periods, we aim to simulate chronic exposure scenarios more accurately.

To validate our model predictions, we propose a comparison with human biomonitoring data collected through the European Information Platform for Chemical Monitoring (IPCHEM). This comparison will assess the predictive capability of our PBK model in estimating internal doses of both DEHP and its metabolite MEHP.

By providing insights into DEHP exposure levels and dynamics, our study contributes to a better understanding of the potential health risks associated with this ubiquitous industrial chemical. Ultimately, these findings can inform risk assessment strategies and regulatory decisions aimed at mitigating DEHP exposure and safeguarding public health.

3.02.P-We179 Chemical Fate and Transport on Mars: Review of Key Processes to Inform Model Development

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There is increasing interest among governments and private companies in sending humans to Mars in the coming decades. This would cause a massive increase in humanity's environmental footprint in the Martian environment compared to the total of 16 uncrewed robotic missions sent to Mars since the 1970s. A recent perspective highlighted the need to expand international requirements around contamination prevention on Mars which currently only cover microorganism contamination to address

anthropogenically-introduced chemical and material pollution. As has been the case for policy setting on Earth, fate and transport modeling will be a key component of chemical and material management on Mars. In this study, we review processes in the Martian environment relevant for chemical and material fate and transport modeling, highlighting key differences with Earth-relevant processes. Examples of factors impacting chemicals include partitioning to dust and dust-borne transport, chemical interactions with CO₂ surface ice and "snow" precipitation (known to occur in the Mars Polar Regions), and extreme temperature gradients in the near-surface environment (on the order of 30 K from the surface to 1.5 meters above the surface) which could impact partition ratios between air and dust at and near the surface.

Furthermore, persistence of chemicals in the Martian environment would likely differ from that on Earth, considering the lack of (known) surface microbial activity, no surface hydrological processes, and a more intense radiation environment (e.g., UV-C). Regarding environmental processes impacting material fate and transport, the presence of UV-C radiation and intense dust erosion processes could result in differential micro- and nano-particle formation from bulk materials compared to Earth conditions.

Furthermore, the lower air density compared to Earth (6 hPa versus 1010 hPa) and the roughly one third gravity on the surface could impact particle long-range transport efficiency compared to Earth. Additional research is needed to improve understanding of these and other processes, prior to their incorporation into regional or planetary-scale fate and transport models. Such models will be key in informing the engineering or other mission requirements that should be incorporated into expanded regulations addressing chemical and material pollution on Mars.

3.02.P-We180 Critical Review of Measured Data and Models for Predicting Chemical Partitioning Properties

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Physical-chemical property data are fundamental to determining chemical exposure and risk assessment. Among the most common partitioning properties required are the octanol-water (KOW), octanol-air (KOA), and air-water (KAW) partition ratios. Reliable physical-chemical property data are required for chemical evaluations and uncertainty is inherent whether the data are measured or modelled. The freely available on-line Exposure And Safety Estimation (EAS-E) Suite platform (www.eas-e-suite.com) contains approximately 85,000 discrete organic chemicals compiled from regulatory programs world-wide. The experimental property data have been curated in this work and pre-calculated predictions from quantitative structure-property relationships (QSPRs) are provided for all chemical in the database. The QSPR packages with predictions are IFSQSAR, EPI Suite, and OPERA. Experimental measurements are only technically possible within a range of values, e.g., log KOW range from about -5 to 11, but QSPR predictions can span many of orders of magnitude outside of these bounds. Possible minimum and maximum values for the commonly used partition ratios are suggested which can be applied to flag QSPR predictions as unreliable. For data-poor chemicals the QSPR predictions are compared individually and as a consensus prediction to the experimental data in EASE Suite. In addition, the applicability domains of the predictions are investigated to determine which data-poor chemicals in the database have the most uncertainty in their predicted values and are in the most need of further research and experimental measurements. Chemicals which are large and are solids have the most uncertain partitioning properties, in addition certain chemical classes and functional groups are identified to have more uncertain properties.

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3.02.P-We181 Long-Range Transport Potential of Volatile Methylsiloxanes: Negligible Transfer and Accumulation in Remote Areas

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The long-range transport potential (LRTP) of chemicals has been evaluated in source regions and remote areas. Since various pathways exist for the fate and transport of chemicals to remote areas without direct emissions, environmental multimedia models are essential for accurate LRTP assessments of different chemical compounds. For the assessment of LRTP of volatile methylsiloxanes (VMS), this study employed the Emissions Fractions Approach (EFA) that enhances the existing OECD Pov and LRTP Screening Tool. The EFA provides LRTP metrics in terms of fractions of dispersion (?1), transfer (?2), and accumulation (?3) in remote surface media, with threshold values recommended based on the assessments of existing persistent organic pollutants (POPs). Realistic emission scenarios for VMS as well as the three basic modes of emissions (100% to air, water, or soil) were evaluated with the model. Since LRTP of VMS is significantly influenced by the emission mode, a sensitivity analysis was also conducted. Due to unique physico-chemical properties of VMS (i.e., high volatility and high hydrophobicity), linear and cyclic VMS are predominantly distributed in air and may travel long distances in air, as indicated by dispersion fraction (?1) exceeding the threshold values. On the contrary, the fractions of transfer (?2) and accumulation (?3) for realistic emission scenarios of VMS are 10-100 times smaller than their maximum values from emission scenarios of 100% to air, water, and soil. More importantly, ?2 and ?3 for realistic emission scenarios are 2-4 and 3-6 orders of magnitude smaller than the respective threshold values, indicating that deposition to the surface media and accumulation of all tested VMS are expected to be negligible. Thus, it is highly unlikely that VMS would accumulate in remote environments.

3.02.P-We182 Seamless Forward Assessment of Toxic Risks in River Networks for Mixtures of Chemicals Originating from Wastewater Treatment Plant Effluents

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Chemicals in the aquatic environment can be harmful to organisms and causing toxic risks to the aquatic ecosystem. A main source of these chemicals are point sources (households and small industries), some are permanently introduced depending on the number of people connected to wastewater treatment plants (WWTPs), other have seasonal concentration differences. Especially at low water situations the toxic risk may increase due to lower dilution.

We test the hypotheses that the accumulated urban discharge fraction (UDF) in a river network is a robust proxy for the toxic risk induced by discharged chemicals. To prove this we used available catchment data like stream network and related WWTPs, the amount of wastewater and discharge data as well as data from a European reference mixture data set containing concentrations of chemicals typical in European wastewater treatment plant effluents. Based on these data we calculated mixing concentrations and toxic units for 80 chemicals, among them pesticides, biocides and pharmaceuticals, besides other typical wastewater-related compounds, such as sweeteners and corrosion inhibitors. Measured data (WFD, 2015 - 2021) at 87 stations in Thuringia were compared with the modelled ones and showed good correlations for pharmaceuticals and no correlation with pesticides. This data supports the identification of different sources of compounds occurring jointly as mixtures in aquatic systems which is important for pollution and risk management.

3.02.P-We183 Predicting the Input of Sunscreen Components into a Recreational Lake

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Sunscreens contain UV filters which vary in physical and chemical properties influencing not only their contribution to skin protection but also their environmental fate and potential toxicity. Common UV filters are titanium dioxide (TiO₂) and zinc oxide (ZnO₂), in nano scale, and organic molecules such as avobenzone, octinoxate and oxybenzone used in commercial sunscreens. These UV-filters are directly released into water bodies, especially recreational lakes, during bathing, where they can exert an adverse effect on aquatic microbial communities. Currently there is a lack of data about the pollution of these filters, especially inorganic ones, from sunscreen in recreational lakes. In particular, small lakes with long water retention times are expected to be especially sensitive to pollutions such as nano TiO₂ because they are not biodegradable and dissolve only under extreme conditions. Therefore, it is urgent to estimate the concentration and input of UV-filters in recreational lakes to facilitate the ecological risk assessment. To address these issues, we modelled the amount of the released UV-filters in typical recreation lake in south Germany by combining information from survey, bathers monitoring, local weather data and experimental determination of the release from the skin (wash-off rate). The collected data were then used to train a predictive model to predict the amount of UV-filters released in the lake depending on the weather and the date and accounting for the adaptive behaviour of bathers. For example, collected data shows 76% of people visiting the study lake (Bassin des Mouettes, Lauterbourg, France) use sunscreens. Preliminary data of wash-off rate experiments indicates the maximum rate for inorganic UV filter, in this case n-TiO₂, was almost 32%. The proposed model is a first essential step to determine the relevance of UV-filter released from bathers for lake ecosystems.

3.02.P-We184 Fate and Transport Modelling of Trifluoroacetic Acid in the Hudson and Cauvery Watersheds Due to HFO-1234ze(E) Emissions from Potential Pressurized Metered Dose Inhalers Usage

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HFO-1234ze(E) is a near-zero global warming potential, next-generation medical propellant in development for use in pressurized metered-dose inhalers (pMDIs). HFO-1234ze(E)-containing pMDIs have a very low carbon footprint akin to a dry powder inhaler. Due to the presence of a CF₃ moiety within its structure, atmospheric degradation of HFO-1234ze(E) is recognised to liberate low levels of trifluoroacetic acid (TFA). To quantify the levels of environmentally formed TFA following HFO-1234ze(E) emissions from future pMDIs, we used TFA deposition fluxes from the global model around the Hudson (USA) and Cauvery (India) watersheds to estimate total TFA accumulation in surface water of these rivers due to 20-years of continuous HFO-1234ze(E) emissions from future pMDI usage. Our detailed watershed modelling predicts that the resultant concentration of pMDI-based TFA in Hudson River subbasins would vary between 0.002 0.005 ppb. In comparison to the Hudson, watershed modelling

over the same period predicts slightly higher accumulation of TFA in surface water of the Cauvery River, varying between 0.009-0.019 ppb. Analysis of TFA mass allocation suggests that the larger increase in TFA accumulation in the Cauvery watershed (which has lower HFO-1234ze(E) emissions than the Hudson) is likely to be due to lower transport of TFA into the oceans (Hudson: 63% of TFA was deposited in ocean; Cauvery: 19% of TFA was deposited in ocean). Using the German Environment Agency's precautionary measure for TFA in drinking water (10 ppb), these results suggest that the Margin of Exposure (MoE) for TFA due to future usage of pMDIs is 2000 in the Hudson watershed and 500 in the Cauvery watershed.

3.02.P-We185 An Attempt at Model-Based Comparative assessment of PFAS Exposure Potential *Monami Kondo, Kazuya Inoue, Shigeki Masunaga and Wataru Naito, National Institute of Advanced Industrial Science and Technology, Japan*

The environmental and human risks of per- and polyfluoroalkyl substances (PFAS) is drawing considerable attention both scientifically and from regulatory perspectives. There are numerous types of PFAS in society, many of which contribute to improving our quality of life. Regulating all these compounds simultaneously is impractical. Although the amount of biomonitoring data on specific PFAS, such as PFOS and PFOA, is increasing, the exposure levels of many PFAS remain unknown. To prioritize which PFAS compounds require risk management in the future, exposure and risk assessments based on realistic scenarios are essential. While a modeling approach could be effective in evaluating the effectiveness of measures such as substance substitution, there are currently few assessments focused specifically on PFAS. Additionally, there are very few studies evaluating the applicability of models, originally developed for persistent organic chlorinated compounds, to PFAS. In this study, we aimed to assess and compare the exposure potentials of PFASs using existing exposure models. We report on the results of the comparative exposure assessment of various PFASs based on model-based approach under specific environmental scenarios. In the presentation, we will discuss the advantages and limitations of the model-based exposure assessment for PFASs, as well as the areas where further research is needed, based on the results of the comparative assessment under specific environmental scenarios.

3.02.P-We186 Investigating Mosquitoes as Bioindicators for PFAS Contamination Across Environmental and Biological Systems

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants resistant to degradation. Their bioaccumulation in ecosystems and biomagnification in organisms pose significant toxicological and reproductive risks to wildlife. In humans, PFAS exposure is increasingly associated with damage to the liver, kidneys, and reproductive systems. The detection of PFAS in human blood serum raises public health concerns; however, comprehensive monitoring is limited by resource constraints and challenges in reaching underserved populations. This study aims to assess the feasibility of using mosquitoes as biomonitoring tools for PFAS exposure, investigating their potential to reflect environmental and biological contamination. We evaluate whether mosquitoes acquire PFAS through blood feeding and examine the contribution of environmental exposure to their PFAS body burden. To distinguish between these pathways, mosquitoes are reared in PFAS-spiked water to adulthood to determine the extent of PFAS uptake from water. A separate colony, reared in uncontaminated water, is fed blood meals spiked with the same PFAS mixture to assess uptake via blood feeding. Field collections of adult mosquitoes from five sites near sewage treatment plants on Maryland's eastern shore were analyzed for PFAS compounds using liquid chromatography-mass spectrometry (LC-MS) based on EPA Method 1633. Preliminary data revealed total PFAS loads ranging from 12.5 to 78.7 ppb across the sites, identifying 14 unique PFAS compounds. PFOS was the most prevalent, with an average concentration of 17.3 ppb (± 2.6). Long-chain PFAS dominated the samples, with three short-chain types also detected. Of these, eight were carboxylates, two were sulfonates, and three were fluorotelomers. Preliminary findings demonstrate that mosquitoes naturally accumulate PFAS, suggesting their potential as biomonitoring tools. By evaluating PFAS uptake through environmental exposure and blood feeding, this study lays the foundation for future investigations into mosquitoes' suitability as bioindicators of contamination in diverse settings.

3.02.P-We187 Helping the Risk Assessor: Improvements to Tools for Environmental Risk Assessments of Metals

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A tiered approach under the European Water Framework Directive has been developed for implementing bioavailability-based Environmental Quality Standards (EQS) for metals. As part of Tier 2, simplified tools to account for bioavailability can be used. bio-met is one of the simplified tools that has been developed for use as part of Tier 2 to assess the potential freshwater risks and compliance with EQSbioavailable for cobalt, copper, nickel, lead and zinc. While Tier 2 provides an opportunity for the use of simplified tools it has certainly been the case that simplified tools have not always been simple or user friendly. To use bio-met, freshwater chemistry data is needed; although large amounts of data are generated by environment agencies, data are not always publicly available or in a format that is easy to use. To remedy this, the EU Physicochemical Database Tool was launched in 2020 containing freshwater monitoring data from European countries to assist practitioners in performing risk assessments. Data are continually being generated; thus, it is important that the database is routinely updated and that the spatial coverage is as extensive as possible to deliver continent-wide assessment of potential metal risks.

Based on feedback from users, bio-met has been extensively updated covering both scientific and useability updates. The applicability range of bio-met has been increased for nickel, zinc and cobalt via updates to the full Biotic Ligand Models (BLMs) and the look-up tables within bio-met. Additionally, an assessment of when bio-met provides outputs has been performed, and the conditions where predictions are provided have been refined. Useability updates have also been integrated to include metal selection, result export functionality, clearer guidance, and a built-in hardness conversion tool.

The EU Physicochemical Database has been updated with an additional 36 datasets, covering 33 European countries, and encompasses over 2,300,000 additional samples (compared to ~1,000,000 after the initial compilation) from more than 84,000 sites. All monitoring data have been reliability and relevance assessed for inclusion in the database.

The updates to both bio-met and the Physicochemical Database have been designed to improve the user experience in applying regulatory guidance, with a goal of increasing useability and ensuring high-quality, evidence-driven conclusions can be made when evaluating the potential risks of metals in European waters.

3.02.P-We188 Dietary Exposure Estimation: A Probabilistic Approach for Exposure Assessment to Pesticide Residues Across Europe

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Accurate dietary exposure assessment is vital for understanding the health implications of pesticide residues in food. Pesticides frequently occur in the foods people consume daily, resulting in constant exposure via their diets. While dietary exposure has been extensively addressed using conservative approaches as part of risk assessment studies, realistic levels of dietary exposure to pesticides among the European population remain underexplored. Furthermore, the presence of unquantifiable pesticide residues in food commodities, which frequently arises from the limitations of analytical methods, detection thresholds, and instrumentation capabilities, results in a substantial portion of residue data being missed. This issue further compounds the challenge of accurately estimating the true levels of dietary pesticide exposure.

To address the above-mentioned gaps and challenges, this research offers a comprehensive probabilistic estimation of human dietary exposure to pesticides, incorporating the complete range of residue concentrations detected in food commodities. This approach, verified against measured pesticide concentrations in duplicate food portions, offers a more accurate representation of pesticide intake among the European population.

To achieve this goal, open-access datasets from the European Food Safety Authority (EFSA) on pesticide residue concentrations and food consumption were utilized. An integrated computational approach was employed to impute undetectable residues (below the limit of quantification). A Monte Carlo simulation framework was then applied to combine the imputed residue concentrations with food consumption data. This produced probabilistic exposure distributions that capture variability across demographic groups and dietary patterns in European countries from 2011 to 2020.

Our approach enhances precision by moving beyond deterministic estimates to probabilistic modeling. It provides useful information not only for regulators seeking a more nuanced understanding of potential

dietary risks but also for epidemiological research and studies requiring the evaluation of temporal or spatial trends in dietary exposure.

3.02.P-We189 A LLM-Based Toolbox for Automated Text Mining on the Uses of Chemicals

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Chemical and material technologies are integral to modern life, offering countless functionalities. However, many chemicals and materials pose risks to human health and the environment. Assessing their safety and replacing hazardous substances with safer alternatives is critical but challenging. Globally, over 350,000 chemical substances are registered for use, yet fewer than 25% have undergone safety assessments. A significant barrier to the assessment and management of chemicals is the lack of accessible and reliable data on chemical uses. Such data are crucial for evaluating potential environmental releases, human exposure, and designing safer alternatives. Current manual approaches to collating such information are time-consuming and prone to omissions. This study presents a novel toolbox leveraging fine-tuned large language model (LLM) to systematically extract and map chemical use data from scientific and regulatory texts. This approach facilitates exposure modeling and improves safety assessment workflows.

The toolbox is built upon a fine-tuned LLM optimized for chemicals. It uses natural language processing (NLP) techniques, including named entity recognition (NER), relationship extraction, and context-aware classification, to identify chemical entities, usage contexts and relevant parameters. The model was trained on a curated dataset of peer-reviewed publications and public databases. Post-processing modules structure the extracted data for downstream exposure modeling applications. Future work will focus on expanding the model's training corpus, improving multilingual capabilities, and integrating predictive features for emerging chemical applications.

3.02.P-We190 Determination of Vial Surface Contamination of Commonly Used Antineoplastic Drugs in Türkiye by LC/MS-MS Method

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The number of people diagnosed with cancer is increasing worldwide. Accordingly, the use of antineoplastic drugs is also increasing. It is known that antineoplastic drugs pose a risk to the personnel working in the process from the production stage to the disposal of their wastes and regulations have been implemented to eliminate this risk. This study aims to determine the levels of drug contamination and cross-contamination on the surfaces of vials containing six commonly used antineoplastic drugs active ingredients in Türkiye: 5-fluorouracil, doxorubicin, etoposide, gemcitabine, ifosfamide, and cyclophosphamide. Samples were taken from the vial surfaces of the drugs by wiping method and analyzed by LC/MS-MS. Based on the analysis results, a statistically significant level of contamination/cross-contamination was detected on the vial surface of the drug with six different active ingredients used in the Turkish market with the active ingredient of the drug and other cytotoxic drug active ingredients originating from the production process. Contamination with the active drug substance was detected in 41 out of 65 samples (63.08%), and cross-contamination with other drug active substances was found in 37 out of 75 samples (49.33%). The highest contamination level was detected on the surface of a 5-fluorouracil vial at 2276.920 ng/cm² / 133199.848 ng/vial. The presence of drug vials in which no contamination was detected indicates that contamination can be prevented to a very high extent if the necessary precautions are taken during the production phase. In order to precisely demonstrate the prevalence of contamination, there is a need to conduct studies with antineoplastic drugs with active ingredients other than the six active ingredients used in the study.

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3.02.P-We191 Aerosol Deposition and Risk Assessment from Humidifier

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Humidifier is widely used in daily life, and the aerosols they generate can enter the respiratory system. This study aims to calculate the mass concentration and regional deposition of aerosols in the respiratory

tract from a humidifier and explore their relationship with 3D cell models for in vitro toxicity assessment. Benzalkonium chloride (BAC), didecylmethylammonium chloride (DDAC), polyhexamethylene guanidine (PHMG), and paraquat were selected as test substances and were dispersed using an ultrasonic humidifier. Aerosol distribution in the 0.01–10 µm range was measured over 60 minutes using scanning mobility particle sizer (SMPS) and optical particle sizer (OPS) in a 0.125m³ acrylic test chamber. Deposition fractions for different respiratory regions were calculated using the multiple path particle dosimetry (MPPD Ver 3.04) model by US EPA, and the deposition mass was derived accordingly. For BAC, the deposition mass in the head, tracheobronchial, and pulmonary regions was calculated as 3.81×10², 1.29×10¹, and 1.04×10⁻¹ µg/cm², respectively. DDAC showed deposition values of 1.14×10⁴, 5.96×10¹, and 4.97×10⁻¹ µg/cm². The largest deposition occurred in the head region and the smallest in the pulmonary region, implying removal of larger particles in the upper respiratory tract (URT). The proposed approach can be used to convert ambient concentration resulted from continuous emission by a humidifier into internal dose at different respiratory tissues, relating to the external exposure concentration and the dose metric used for in vitro test assays.

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3.02.P-We192 Enabling Improved Worker Exposure Modeling During Powder Handling Operations

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Dust generated during powder handling is a major worker exposure risk. The "dustiness" of a powder its tendency to become airborne is key in determining exposure levels. Current exposure models depend on dustiness estimates or measurements, but obtaining accurate dustiness measurements is often time-consuming, leading to overly conservative exposure predictions.

To address this, we report the first development of an in silico dustiness prediction model which is interoperable with existing exposure or risk assessment models. The model estimates the relative change in the dustiness with the change(s) in physiochemical properties of a powder and the energy applied during its handling. The first results from testing the model with eight commercial powders showed both mass-based and number-based dustiness estimates to be generally underestimated, with experimental values mostly falling out of the 95% confidence interval. However, when used with exposure models (e.g., ECETOC-TRA, Stoffenmanager, ART), these estimates led to more accurate, less conservative exposure predictions. Though promising, the model currently offers semi-quantitative estimates and requires further development for absolute dustiness quantification, specifically to refine/alleviate some of the underlying assumptions about powder flowability and interdependencies between powder properties.

Aligned with a tiered approach, the model supports safe-by-design principles from early innovation stages, allowing users to optimize powder and process parameters to reduce exposure. When developed as a fully quantitative model, future prospects can include integrating it with PBPK or MPPD models to translate external exposure levels into internal organ concentrations.

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3.02.P-We193 Assessment of PM1-Bound PAH Levels in a Primary School: A Comparative Study of Mechanical and Natural Ventilation

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Indoor air quality in primary schools significantly impacts children's health due to their physiological vulnerabilities and extended indoor exposure. This study investigated PM1 and PM1-bound PAH concentrations in two primary school classrooms - one with mechanical ventilation (MV) and one with natural ventilation (NV) - in an industrial area exposed to vehicular emissions and residential combustion. PM1 samples were collected using Harvard impactors during 50 days of the fall academic term, with PAH analysis performed via GC/MS. Results showed that MV maintained more stable PM1 levels (2.75-25.5 µg/m³) compared to NV (2.17-198.2 µg/m³) and outdoor environments (1.39-182.5 µg/m³). Total PM1-bound PAH concentrations were highest outdoors (9.30 ± 8.29 ng/m³), followed by NV (3.93 ± 4.57

ng/m³) and MV (2.57 ± 1.76 ng/m³). Naphthalene and benzo[a]anthracene were the predominant PAH compounds. CFD analysis revealed optimal ventilation performance at 30° diffuser angles. The study demonstrates that mechanical ventilation effectively reduces PM1 levels and recommends upgrading to F9 filters for enhanced PM1 capture while highlighting the importance of diffuser angle optimization for uniform air distribution.

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3.02.P-We194 Estimating Global Spatially Resolved Marine Emissions and Exposure to Down the Drain Chemicals

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Domestic wastewater discharged to the environment may contain chemical substances that are the result of human activity. While many discharges are to freshwater rivers and lakes, some facilities in coastal areas discharge directly into the marine environment. However many down-the-drain exposure models assume 100% of the chemical mass discharged from STPs or untreated populations is released into the freshwater environment. A dataset was developed to understand what fraction of the population in coastal areas are served by STPs that discharge directly to a marine environment. Information on STP location, population served, and effluent flow were collected for 44,000 STPs across 34 countries serving almost 800 million people.

Chemical substances are also discharged into freshwater which then may travel through the hydrological network to the marine environment. Hydrologic travel distance/time for each inland STP as well as untreated mass was developed along with estimated aquatic half-lives, to estimate discharges into rivers moving to coastal waters. The developed emission model is a mass balance model.

The third phase of the study developed two exposure models for 3436 regions covering 75 coastal countries to generate Predicted Environmental Concentrations (PECs). The Moving Prism Model is a steady-state box model that calculates a PEC by advecting a triangular wedge of coastal water past a chemical discharge point, with the speed of advection being determined by net ambient current speeds using bathymetry data, long term (365-day average) scalar current speeds and near coast hydrodynamics. The Mixing Zone Model is an alternative steady-state box model that calculates a PEC should a chemical be discharged into still water (i.e., with no ambient current speed). Some initial comparisons to monitoring data show good agreement.

This work shows that it is possible to use country-based datasets including geo-referenced STP and wastewater discharge types to refine emissions estimates for use in environmental exposure models and risk assessments at a refined sub-country spatial scale.

The exposure model provides a screening level, globally-consistent approach for assessing the potential impact of down-the-drain chemicals on the marine environment. These models generate estimates of Predicted Environmental Concentration and represent an intermediate level between generic, multi-media models and detailed, data hungry process-based models.

3.03.A Measuring, Modelling and Monitoring the Environmental Behaviour and Exposure of Pesticides

3.03.A.T-01 SPOTMOD: Modelling the Reduction Effect of Spatially Distributed Spot Applications on Pesticide Runoff Losses with a 2D Probabilistic Framework

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Field spot treatments of agrochemicals, e.g., by automated weed detection in combination with precision application technology, can significantly reduce chemical application rates in the field. Localized, high-resolution chemical application reduces off-field drift and surface runoff to adjacent water bodies and terrestrial habitats. While quantification of drift reduction is advancing, very limited work exists for the quantification of potential pesticide runoff reduction from spot application practice in the regulatory context.

Particularly for crops with dense surface cover (e.g., cereals), the untreated field area can act as a sink for

dissolved and adsorbed pesticides before the surface runoff exits the field. Part of the difficulty in quantifying the potential reduction of pesticide runoff stems from the (quasi-)random pattern of spot applications on the field during each crop season and across years during long-term regulatory assessments. A new 2D mechanistic-probabilistic modeling framework, SPOTMOD, has been developed that considers (1) sources: pesticide runoff generation from treated spots in the field, (2) sinks: infiltration, sedimentation and pesticide trapping on downslope segments (after spots) of the field, (3) variable vegetation conditions during the cropping period and their effects on infiltration and sedimentation, (4) variable spot size and treated area fraction, (5) variable spatial application patterns (random, quasi-random and observed), (6) long-term exposure calculations (PEC_{sw}). In a first step, an application pattern on the field is generated based on spot size and treated area fraction. The field is then divided into strips in slope direction with the same width as the treated spots. These strips are subsequently simulated with a coupled PRZM-VFSMOD modelling system, which can deal with complex sequences of treated spots (sources) and untreated areas (sinks) along the flow path. For each runoff event, the edge-of-field runoff hydrographs and associated sediment and pesticide loads are summed up and fed into a TOXSWA metamodel. Different realizations of the application pattern allow to construct a probability distribution of PEC_{sw} for each event and thus identify realistic worst-case application patterns. Parallel optimization of SPOTMOD on a High-Performance Computer allows for systematic exploration of key settings. An end-user solution around a metamodel created from millions of runs is also developed for quick assessments.

3.03.A.T-02 Harmonised Framework for the SETAC Spatially Distributed Leaching Modelling of Pesticides Initiative: 2025 Update

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Spatially Distributed Leaching Modelling (SDLM) of pesticides is a methodology to estimate the leaching potential over a large spatial extent such as national or European scale. SDLM can help to put groundwater monitoring programs into context. It is described in the FOCUS groundwater report and foreseen to be used as a higher tier leaching risk assessment. SDLM is already used as a higher tier assessment in the national authorization procedure in some EU countries and will probably become more important in the future. At the SETAC Europe 2020 online meeting, the initiative was officially formalised as a SETAC working group, consisting of a triad of members from regulatory agencies, academia, and the private sector. The main products of the working group will be a harmonised modelling framework including the data needed to run these models, and documents describing the use of the framework in regulatory assessments. The framework will serve two different tiers of the groundwater risk assessment scheme, i.e., Tier-3b and Tier-4. This year we will present final results for both tiers of the risk assessment scheme.

At Tier-3b, the leaching models PEARL and PELMO were used to calculate the 80th-percentile of the leaching concentration at 1-m depth in each FOCUS zone. The models were run for approximately 10,000 scenarios. These scenarios are unique combinations of land-cover, climate and soil data. Results show that the leaching concentration simulated at Tier-3b is generally lower than the leaching concentration simulated for the FOCUS scenarios at Tier-1 of the groundwater assessment scheme. This means that the FOCUS scenarios are sufficiently conservative and that an update of these scenarios is not necessary. At Tier-4, SDLM can be used to set monitoring sites into context. For this purpose, simulations will be carried out with PEARL and/or PELMO for each of the monitoring sites. In these simulations, site-specific soil, crop and climate data will be used. The simulated leaching concentration will be plotted at the cumulative frequency distribution of the leaching concentration simulated with SDLM to show if the monitoring sites represent vulnerable conditions. During the presentation at the SETAC 2025 Annual Meeting, a real-world example will be shown.

3.03.A.T-03 Using Different Spatial Layers to Derive pH Values for Modelling: A Critical Review of EFSA's Spatial Dataset

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Regulation 283/2013 mandates the investigation of chemical fate and behaviour in soils across three pH ranges (pH 5-6, 6-7, and 7-8 in CaCl₂ solution) to assess the potential of pH dependency. When pH-dependent behaviour is identified, it must be incorporated into the risk assessment. However, current guidelines lack standardised methods for deriving modelling parameters in that case. For this reason, a spatial analysis of the available geodata layers of pH in Europe is presented (EFSA 2012, ESDAC/JRC 2019), to assess their accuracy against real soil measurements (LUCAS 2018). This work aims to check the robustness of the 10th- and 90th-percentile estimations of pH on arable land, using the EFSA spatial dataset, proposed as an option to estimate substance properties in the risk assessment. The results showed it was possible to replicate the values suggested by EFSA 2020 at the EU scale (EU: 5.1,6.5,8.0) but not the figures provided by UBA at the regulatory zone level (Northern: 4.4,6.2,7.5; Central: 5.1,6.5,7.8; Southern: 5.4,6.9,8.0). This suggests a methodological inconsistency in the derivation of endpoints, which needs to be addressed before guidance on pH dependency is noted. The work also indicated that changing the geodata layers used in the analysis affects heavily the derivation of modelling endpoints. It demonstrated, once again, that the EFSA spatial dataset has the worst fit vs real data, compared to any other geolayer available at the moment in Europe ($R^2 = 0.32$ of EFSA 2012 vs $R^2 = 0.61$ of ESDAC/JRC 2019). The comparison with other geodata layers also suggested conceptual flaws in the use of categorical values (i.e., median of the pH distribution for a soil) applied to extended regions, as done in EFSA 2012, for statistical purposes. Given that the EFSA spatial data are a cornerstone in several recent guidance developments, the outcomes of this work highlighted how discussing the quality of geodata is not only a technical issue of the GIS community but has far-reaching regulatory implications in e-fate. Therefore, the authors urge a broader scientific discussion around the use of GIS layers in risk assessment of plant protection products, to reduce the gap between regulatory requirements and agronomical realities, by considering the most recent advances in soil mapping in Europe and setting a version control group like the FOCUS one

3.03.A.T-04 Conduct and Use of a Tier 4 Monitoring Programme in the FOCUS Regulatory Framework

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Groundwater (GW) monitoring is the highest tier (Tier-4) in the leaching assessment of plant protection products in Europe, providing a realistic exposure assessment, and, if well-performed, can be used to prove safe uses in risk assessments. Given a current lack of European guidance, uncertainties remain about Tier-4 interpretation, deterring broader implementation of monitoring in the regulatory process.

Nonetheless, uncertainties can be addressed scientifically and pragmatically, to reduce reliance on arbitrary safety factors which deviate from established FOCUS framework approaches. An open scientific discussion on FOCUS Tier-4 data assessment is presented using data from an in-flight pan-European programme. The rationale behind this work is to gain a robust and realistic understanding of environmental GW concentrations that maintains the consistency of the FOCUS scheme across tiers. The study, conducted since 2015, collected thousands of samples from 70 edge-of-field sites in vulnerable cereal regions across the EU. The network assessed pinoxaden and its metabolites in shallow GW under actual use conditions and practices. Sites were selected in vulnerable regions that also had cereal cropping and shallow GW, aiming for an approximate 90th overall percentile of vulnerability. Various uncertainties identified post-initiation, including hydraulic connectivity, GW flow variability, and differences between intended/actual uses, were subsequently addressed during the life of the programme. Thus, to maintain consistency across FOCUS tiers, the 80th percentile of the annual average concentration was calculated, along with the 90th overall percentile of all the samples in each regulatory zone (using both original concentrations and those normalized to account for different application rates and frequencies). This specific pinoxaden programme demonstrates that interpretation of monitoring data for the FOCUS Tier-4 assessment can be robust and scientific, and can be made sufficiently vulnerable through purposeful design choices. Targeted, conservative monitoring programmes represent a robust and realistic approach to data generation that is coherent within the tiered FOCUS framework and add evidence to support and prove safe uses at the European level. This approach reinforces scientific robustness and public confidence in a well-established tiered risk assessment approach that includes environmental GW monitoring in risk assessment and management decisions.

3.03.A.T-05 Steps Toward a Re-validation of Methods and Models Used in the Risk Assessment of Plant Protection Products in the Light of Climate Change

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Many methods and models used in the risk assessment of plant protection products (PPP) (under Regulation (EC) No 1107/2009) were developed more than 20 years ago, describing the environmental and agricultural conditions at that time or prior to that time. The project had the objective to identify climate-sensitive parameters and processes, perform a gap analysis and define work packages to enable the Nordic zone to reevaluate and possibly adapt the current risk assessment to be fit for purpose in the coming decades.

Responsible authorities of member states (MS), industry, and research institutions were contacted about relevant projects. Their feedback was evaluated and summarised. A list of climate-sensitive parameters and procedures used in the risk assessment was compiled and prioritised for importance or impact. To identify the direct impact of climate data on Predicted Environmental Concentrations in groundwater (PEC_{gw}) an impact assessment of two climate projections for the FOCUS PELMO Hamburg scenario was performed for nine test substances with varying soil DT50 values and KFOC values. A gap analysis was performed to identify the information lacking to assess whether the climate-sensitive parameters used in the risk assessment are still representative, in which way they are expected to be changed, and to specify further work and specific tasks that need to be performed to conclude on these points.

The direct impact assessment of changed weather data in PELMO yielded that PEC_{gw} values decreased with increasing temperature despite increasing rainfall. Temperature changes enhance degradation and evapotranspiration, while precipitation changes increase leaching. Changes in rainfall intensity were not considered in this exercise.

Indirect impact is expected from changes in soil microbial communities due to drought and flooding, shifts in agronomic dates or an extension of agricultural land further north into potentially vulnerable areas. Further it is expected that new management practices will become more important. This may include precision farming techniques like application via drones and spot applications, and mitigation options such as micro-dams or conservation tillage.

Prioritised work packages were identified for the authorities in the Nordic zone including literature reviews, updates and development of guidance, updates of weather files and agronomic dates in FOCUS, as well as re-evaluation of the relevance of FOCUS crops and scenarios.

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3.03.B Measuring, Modelling and Monitoring the Environmental Behaviour and Exposure of Pesticides

3.03.B.T-01 EFSA Drinking Water Treatment Guidance – Approaching the Environmental Exposure Assessment

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The new EFSA guidance document (GD) on drinking water treatment assessment will be applicable for plant protection products (PPP) active substance (a.s.) (re)approval dossiers submitted on or after 1 April 2026. It is applicable to all uses with emissions to groundwater (gw) and/ or surface water (sw) for the a.s. and all metabolites listed in the residue definition for gw and sw. Applicants are facing tight timelines and high workload due to the overall complexity of the GD and the additional requirements for the environmental exposure assessment (EEA). In this presentation, we will discuss the impact of the GD on the EEA based on first experience and conclude on further recommendations.

The EEA follows the FOCUS modelling framework. The predicted environmental concentrations (PEC) are compared against a trigger value of 0.1 µg/L. Any compound exceeding the trigger in gw and/ or sw, needs to be assessed further, and the related PECs are directly used in the subsequent assessment steps. For gw, the modelling results are used directly for comparison. For sw, the GD proposes to use the 4-day time-weighted average (twa) PEC_{sw} and additionally, the application of dilution factors which vary depending on use type and EU regulatory zone.

First experience with the GD shows that complexity and required work increase for both, gw and sw modelling. For gw modelling, now, toxicologically non-relevant metabolites need to be assessed further. For sw modelling, many metabolites that were so far only assessed at FOCUS Steps 1 and 2, now require extensive simulations at FOCUS Steps 3 and 4, and the implementation of complex degradation pathways can be challenging. Additional refinements and mitigation measures are required, e.g., restrictions in application rate, frequency or timing, use restrictions related to soil properties, additional buffer zones, refined modelling input parameters, or higher-tier assessments, e.g., GIS based approaches to derive lower

PECs or higher dilution factors.

The EEA according to the EFSA GD requires substantial additional time, work, computational resources, and costs. The challenge is to balance different options, for the EEA as well as for the overall assessment. Comprehensive preliminary EEA can support decision making when developing robust strategies. In this context, it is highly recommended to include the whole product portfolio and if relevant, the biocidal uses of the a.s. to establish overall worst-case concentrations.

3.03.B.T-02 Photolysis in PELMO: Conceptual Review on the Implementability of Scientific Guidance on Photo-Transformation Products in Groundwater

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Soil photolysis is a critical degradation pathway for the environmental fate of pesticides, particularly in surface soils under sunlight exposure. The European Food Safety Authority (EFSA) has emphasized the importance of incorporating photolytic transformation processes in pesticide fate models to improve groundwater risk assessments, especially considering photolytic metabolites. This study evaluates the implementation of soil photolysis within the FOCUS PELMO model, emphasizing parameter sensitivity and environmental influences.

Using the offered EFSA guidance (2022) case study, the analysis examined how parameters like photodegradation half-life (DegT50photo), adsorption coefficient (Kfoc), and precipitation volume and timing affect predicted environmental concentrations (PECs). The model assumes photolysis acts only on substances remaining on the soil surface, with rainfall transporting compounds to deeper layers and reducing photolytic effects. The sensitivity analysis reveals how delayed and lower rainfall increases cumulative photolysis, while higher precipitation volumes or earlier rainfall reduce degradation by shielding substances from sunlight.

The results underscore the importance of considering photolysis in environmental fate models for pesticides and their metabolites, particularly for substances with high Kfoc and extended surface half-lives in soil (DegT50bio). By accounting for dynamic environmental variables like radiation and precipitation, this study demonstrates the need for robust modeling frameworks to support regulatory decision-making and mitigate environmental risks associated with pesticide use.

3.03.B.T-03 Leveraging Irradiated Water-Sediment Study for Environmental Modelling and Exposure Assessment

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Environmental fate (efate) studies of plant protection products (PPPs) for regulatory purpose often require the separation of abiotic and biotic factors. As a higher-tier option under Regulation (EC) No 1107/2009, the irradiated water-sediment study is one of the few exceptions and offers a feasible study design in exploring the real-world interplay between aqueous photolysis and microbial degradation. Despite the existing OECD TG 308 on (an)aerobic transformation in aquatic sediment system and OECD TG 309/316 on aqueous mineralization/photolysis, no formal testing guideline exists for this study type, leaving high flexibility on the study design, but also substantial concern on the relevance and reliability for its regulatory use in efate. Here, we aim to reduce the uncertainty of using this study type as a higher-tier option in regulatory context. As irradiation is the key element distinguishing it from OECD TG 308, we firstly set up a realm of representative EU irradiation scenarios to aid the experimental design on source of irradiation. In a second step, we reviewed existing irradiated water-sediment studies with varying substance profile, experimental design and source of irradiation. Although degradation is often expected to be faster in this study type than OECD TG 308, less is known when it is compared to irradiated OECD TG 309/316 study types. Thus, we also evaluated the impact of this refinement option on degradation half-lives and metabolite profile compared to the other study types and identified the main drivers for change of degradation behaviour. Special attention was paid to evaluate the reliability of using this study type in persistence assessment as well as to derive realistic worst-case modelling endpoints for (photo-)metabolites compared to tests conducted in the dark. Overall, we aimed to offer recommendations from lessons learnt on this study type for a robust design of fit-for-regulatory-purpose irradiated water-sediment studies.

3.03.B.T-04 Field Test of the TOXSWA Pesticide Fate Model: Comparison of Simulated and Observed Linuron in Water and Sediment in Ditches with Stagnant and Flowing Periods

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TOXSWA (TOXic substances in Surface Waters) is a numerical model describing pesticide behavior in an edge-of-field waterbody. Despite its widespread use in predicting exposure for regulatory risk assessments

of aquatic ecosystems, few evaluations of the TOXSWA model's performance using field data have been conducted. In this study, we explore the applicability of TOXSWA for predicting the fate and behaviour of linuron, a highly water-soluble pesticide with low sorption capacity. The experimental observations were obtained from an outdoor ditch study which includes both stagnant and flowing periods and involves three applications. Water samples were taken at five distances from the inlet of each ditch, and sediment samples were taken at four layers of depth. We assess TOXSWA's capability to accurately simulate the spatial (both horizontal and vertical) and temporal dynamics in water and sediment with this case study. An initial parametrization based on data from field observations, lab-measurements and literature captured the general patterns of the observed concentrations in time and space. However, the model underestimated the height and timing of the peak concentration in the sediment, particularly for deeper layers. Therefore, we carried out several calibrations in which various parameters were optimized, including the half-lives for degradation in water and sediment and the diffusion coefficient in (pore)water. This led to an improved fit to the observations, but with an unrealistic parametrization. We hypothesize that a transport mechanism other than diffusion in porewater may be responsible for downward movement in the sediment, for example bioturbation. To investigate this, we are currently modifying TOXSWA to introduce a separate diffusion coefficient for the sediment. We expect that optimizing this diffusion coefficient will result in an improved fit with a plausible parametrization.

Based on these preliminary results, we are confident that TOXSWA will be able to adequately predict linuron behaviour in outdoor ditches in both the water and the sediment layer, capturing temporal changes through periods with varying flow regimes and multiple applications.

3.03.P-Mo172 Effect of Soil Particle Size and Organic Matter on Pesticide Sorption Behavior in Agricultural Soils

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Intensive agricultural practices heavily rely on land exploitation and high inputs of pesticides to produce crop monocultures. Some applied compounds persist in soil, whereas the more mobile pesticides run off or leach through the soil profile and enter surface and groundwater sources. While the overarching mechanisms of pesticide sorption to soil constituents are acknowledged, there remains a lack of understanding regarding the relative contributions of specific soil size fractions and their associated organic matter.

To address this gap, and to discern how the physical characteristics of the soil fractions and their organic matter content contribute to pesticide retention, our research investigates the differences in pesticide sorption between coarse and fine soil size fractions with and without organic matter. Using wet sieving, we first fractionate the soil into the coarse ($> 53 \mu\text{m}$) and fine ($< 53 \mu\text{m}$) soil physical fractions. After removing organic matter from soil size fractions, we obtain the coarse and fine mineral fractions. We perform batch sorption tests to determine the efficiency of 12 pesticide removal from soil solution using separated and treated soil fractions. Our approach allows a comparison between the effects of organic matter and mineral fractions, providing a clearer understanding of the contributions of each component to the overall sorption process.

Our hypotheses are: (1) separated soil fractions with intact organic matter will perform better in pesticide sorption than mineral fractions; (2) the extent of pesticide sorption efficiency will be different between the coarse and fine soil fractions; (3) we will be able to identify patterns regarding the most efficient fractions for the studied compound sorption. The findings of this research provide new insight into the effect of soil properties in reducing pesticide runoff, leaching, and subsequent environmental contamination and thus have important implications for choosing agricultural land-management practices (tillage, animal manure application, etc.).

3.03.P-Mo186 Challenges for Soil Photolysis Modelling in Groundwater Risk Assessment: An Analysis of Surface Layer Dynamics in FOCUS Models

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The European Food Safety Authority (EFSA) guidance on soil phototransformation products in groundwater (GW) recently mandated the use of a soil photolysis module within FOCUS PELMO. Initial tests indicate that the extent of photolysis in FOCUS PELMO simulations is highly sensitive to environmental processes governing the duration for which chemical mass is retained within the photolytically active topmost one millimeter of soil. Notably, this guidance was implemented despite EFSA's acknowledgment that FOCUS GW models were not developed nor calibrated to accurately predict chemical fate and environmental processes in the surface layer.

This study analyses water, soil, and compound behaviour within the surface layers of FOCUS PELMO and compares it to the predicted behaviour in FOCUS PEARL. As the photolysis module has not been implemented in FOCUS PEARL, precluding direct comparison of photodegradation between the models, we assess models' susceptibility to photolysis by analysing the residence time of substances in the surface layer, i.e., the exposure to photodegradation. In the absence of true validation for either model against environmental data in the topmost layer of soil, this analysis provides model cross validation.

A direct comparison of the two models reveals that while soil temperature correlates strongly ($R = 0.85 - 0.94$), soil moisture ($R^2 = 0.07 - 0.33$) and water fluxes ($R^2 = 0.33 - 0.83$) differ substantially. The hydrological disparities between the models result in differences in substance residence time in the soil surface, significantly impacting susceptibility to photolysis.

We conclude that implementing a photolysis module within PEARL would likely yield PECs that differ significantly from those produced by FOCUS PELMO. Thus, both models will require calibration at the topmost soil layer similar to that performed at 1m depth. This therefore raises concern regarding the reliability of risk assessment decisions based on the current in force guidance.

3.03.P-Mo195 Global Pesticide Application Data as Input for Assessing Ecosystem Exposure and Impacts

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Agricultural pesticide applications significantly impact terrestrial and aquatic ecosystems, yet comprehensive data on actual field applications remains fragmented. We address this knowledge gap by developing the first global dataset of agricultural pesticide applications at country-crop-chemical level, incorporating detailed information on crop growth stages, application timing, and methods. This dataset aims to support the development of approaches that can predict how different application scenarios influence exposure and impact patterns across species and trophic levels in terrestrial and aquatic ecosystems.

By synthesizing field-level application records from agricultural monitoring programs (2016-2020) and employing machine learning approaches including XGBoost and conformal prediction, we created a comprehensive dataset on pesticide application, including a spatially-explicit mapping at 5×5 arcminute resolution for each country-crop-chemical scenario. Our methodological framework integrated different datasets into a consistent format capturing application parameters, environmental conditions, and agricultural practices.

The resulting database encompasses more than 2.9 million tonnes of annual average pesticide use across 180 countries and 130 crops, involving 1077 distinct compounds. Four compounds dominate global usage: glyphosate (0.64 million tonnes), sulphur (0.17 million tonnes), mancozeb (0.16 million tonnes), and atrazine (0.1 million tonnes), with China, USA, and Brazil accounting for 46% of total mass application despite covering only 25% of global crop treated area. To address data gaps, we developed estimations for 81 nations, predominantly in sub-Saharan Africa, and supplemented missing application details for over 50% of country-crop-chemical combinations. The dataset provides compound-specific application patterns, including detailed timing and methods, exemplified by Acephate usage in US cotton and soybean production.

This comprehensive resource enables reconstruction of exposure scenarios, identification of chemical accumulation hotspots, and assessment of spatial and temporal exposure variations. The dataset uniquely supports development of targeted risk mitigation strategies and evidence-based policies for protecting biodiversity from chemical stressors, directly contributing to objectives such as the EU Zero Pollution Strategy and Biodiversity Strategy.

3.03.C Measuring, Modelling and Monitoring the Environmental Behaviour and Exposure of Pesticides

3.03.C.T-01 Exposure Route Assessment for Non-Target Arthropods in Ecotoxicity Testing and Field Residue implications

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The first Tier of the pesticide environmental risk assessment for non-target terrestrial arthropods (NTAs)

has a data requirement on effect studies with two standard test species involved in pest control, using contact exposure to dried spray residues on glass plates. The glass plate residue is considered a worst-case exposure scenario compared to the exposure that NTA experience after a downward spray event in the field via contact with residues on (3D-structured) natural substrates, such as plant leaves and soil. This exposure scenario may still underestimate the dietary intake from food residues (e.g., in plant material for herbivorous NTA and prey material for predaceous NTA), as well as direct exposure to the spray droplets during pesticide-application (and spray drift), as discussed in the EFSA Scientific Opinion on NTA risk assessment.¹ This study aimed to follow the chemical accumulation in NTA over a 1 week period using different exposure routes. A single (non-toxic) spray dose was applied using a Potter tower spray system to expose NTA via (1) direct insect overspray, (2) a dried residue on natural substrate surface (leaf or soil), (3) dietary intake of oversprayed food items. A binary mixture of two strongly sorptive herbicides (diflufenican - and prosulfocarb) was tested on larvae of two NTA species: *Mamestra brassicae* (herbivorous moth caterpillar, pollinating species as adult) and *Coccinella septempunctata* (predatory ladybird). Diflufenican accumulation in moth larvae via dietary intake was higher than via natural substrate contact alone. The more volatile prosulfocarb showed 3-10x lower residue unit dose (RUD) than the co-applied diflufenican for each exposure route in moth larvae. Ladybirds showed several deviating accumulation trends compared to moth larvae. Exposure via overspray, leaf contact and particularly contaminated food items, resulted in a relatively higher accumulation of prosulfocarb in ladybirds compared to moth larvae. For both species RUD for several exposure routes were above the 90-percentile RUD values measured in field studies. Topical overspray exposure on the short term, and dietary intake during most of the 7 day exposure tests, resulted in higher pesticide accumulation in NTA compared to contact with natural substrate surface residues. Based on these findings we suggest to take additional exposure routes into account in NTA risk assessment, while differentiating between organism groups.

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3.03.C.T-02 3D Spray Drift Exposure Data for Non-Target Terrestrial Organism Risk Assessment
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The Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) is currently under revision. A more sophisticated risk assessments for non-target terrestrial plants (NTTPs) and arthropods (NTAs) needs a better understanding of actual exposure in off-field areas, especially concerning the quantity and distribution of spray deposited on plant surfaces downwind of treated fields. Key factors influencing plant exposure include drift profiles, spray deposition efficiency, distance from the application site, and environmental conditions, including nearby vegetation like hedgerows.

This study aimed to establish a robust experimental protocol for measuring plant exposures in real-world settings, conduct a field trial, and analyze data to inform regulatory assessments for NTTPs and NTAs. Sodium fluorescein was sprayed on a field of cut rye grass, which had been prepared three days prior, leaving a 20 m uncut strip on the northwest edge for wind considerations. The experimental layout included three replicate treatment plots, each receiving separate spray applications. Samples were collected at various distances from the sprayed area to assess drift penetration, using horizontal chromatography paper, vertical stainless steel rods, and above-ground vegetation quadrats. Meteorological conditions were monitored throughout the process.

Analysis of the spray liquid revealed that airborne spray collected by rods was significantly higher than that on horizontal papers but decreased more rapidly with distance, indicating the filtering effect of the vegetation. The rods showed varying penetration of spray drift droplets, with capture declining from the surface downward. A clear trend of reduced spray deposits with increasing distance from the treated area was evident. Strong correlations between collector types indicated relationships between airborne and sedimenting spray measures. Wind speed was an important factor but did not significantly affect spray deposit results across different runs.

A good correlation between deposits on artificial collectors and vegetation will allow further exploration of collection efficiencies of different plant species, and simplification of experimental protocols. Further refinements to the methodology explored here should consider incorporation of techniques for characterisation of vegetation to support development of flexible modelling representations of penetration, capture and deposition of spray drift within margin habitats.

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3.03.C.T-03 Comparison of Simulated and Observed Downwind Deposits of Spray Drift in Orchard Crops Across Europe

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EU Regulation 1107/2009 requires assessing pesticide exposure for several groups of non-target organisms. Spray drift deposition, a highly variable and significant exposure route, has not yet been reliably estimated at the European level. This variability in exposure arises from differences in crops, crop height, agronomic practices and agro-environmental conditions. Currently, the EU employs the Ganzelmeier-Rautmann drift deposition curves for regulatory environmental exposure assessments. However, these curves, based on experiments conducted in Germany, may not adequately represent the diverse meteorological and crop conditions across Europe. Modelling offers an alternative to experimental drift curves for regulatory purposes, yet no ready-to-use model exists to predict spray drift depositions for upward and sideways sprayed crops at the EU level. The statistical model SPEXUS, originally developed for Dutch apple orchards, reflects conditions in the Netherlands but can be adapted for other fruit crops and climate zones by integrating crop development stages and local weather conditions. The broader model's applicability was tested by comparing the results of SPEXUS to six EU datasets, covering spray drift experiments in pome fruit, citrus fruit and vines, across four countries in two EU regulatory zones (Central, South). These datasets were kindly provided by research groups in Europe. For these data, regression models were developed, resulting in spray drift curves for different crop groups and growth stages. These regression models were compared to the SPEXUS model and the Ganzelmeier-Rautmann curves. Comparisons showed that SPEXUS predictions differed from experimental deposits but aligned with the model's basic assumptions about meteorological conditions and deposition processes. In contrast, the Ganzelmeier-Rautmann curves both underestimated and overestimated the measured drift deposits, while overestimation was the most observed outcome. This implies that for upward and sideways sprayed crops the use of Ganzelmeier-Rautmann curves is a conservative approach from a regulatory perspective, thus defensible. Still, a more advanced model like SPEXUS offers clear advantages in describing spray drift by accounting for variable weather, application techniques and crop types and structures. Further development of such models represents the most promising path towards an EU-wide framework for spray drift predictions in upward and sideways sprayed crops.

3.03.C.T-04 Generating Field-Realistic Predictions of Exposure for Off-field Soil Organisms Using a Spatiotemporally Explicit Modeling Approach

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Natural and semi-natural habitats of soil living organisms in cultivated landscapes can be subject to unintended exposure by active substances of pesticides used in adjacent fields. The European Food Safety Authority released a scientific opinion in which spray-drift and runoff are identified as the most relevant potential exposure routes of off-field soil organisms. It outlined a first approach to estimate off-field soil exposure, designed closely to the FOCUS surface water model approach. This approach assumes independent conservative estimates on local spray-drift and runoff entries and adds them at a single location. This means that 100% of individuals in a population occurring 'off-field' in a cultivated landscape receive worst-case exposures. This does not consider real-world variability of exposure in space and time. These two risk dimensions and their scales are essential when assessing effects and risk according to Specific Protection Goals. We developed a model (xOffFieldSoil, built on the xLandscape platform) and associated scenarios to estimate exposure of off-field soil habitats at flexible levels of realism. The modular approach consists of components each addressing a specific aspect of exposure processes. These components are based on established models in regulatory exposure and operate at scales ranging from local edge-of-field to large landscapes. Presented scenarios start with a schematic edge-of-field situation and extend to real-world landscapes of up to 5x5 km. xOffFieldSoil represents an initial spatiotemporally explicit approach to assess exposure/risk of off-field soil organisms due to spray-drift and runoff entries. The approach is flexible and able to operate at any desired spatial and temporal resolution. Study results are intended to inform the scientific discussion on design of off-field soil exposure, effect and risk characterization approaches in a tiered RA scheme and support the development of Assessment Endpoints for regulatory risk assessment, while the impact of risk mitigation options can be analyzed in detail. The modular architecture of xOffFieldSoil model allows the use of individual process components of different complexity (and reality) levels and validation status. The presented xOffFieldSoil model, scenarios and case study can inform more realistic risk assessment for off-field soil organisms. xOffFieldSoil is publicly available on GitHub <https://github.com/xlandscape/xOffFieldSoilRisk>.

3.03.P-Mo198 Occurrence, Spatial Distributions, and Influencing Factors of Neonicotinoid insecticides in Soils from Agricultural Farmlands in China: A National Study

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Neonicotinoid insecticides (NEOs) are widely used in crop production in China, raising concerns about their health and ecological risks due to persistent residues in agricultural soils. Previous research has reported NEO contamination in soils of China's coastal regions, but national-scale data and information on NEO metabolites remain limited. On the other hand, both NEOs and microplastics (MPs) are commonly found in agricultural fields, but their co-occurrence characteristics under realistic fields have not been reported.

This study found that at least one NEO was detected in all soil sample. Imidacloprid was the most common parent NEO, with imidacloprid-urea as its primary metabolite. Results showed higher NEO concentrations in coastal areas at similar latitudes compared to inland regions. The extent of agricultural film cover (i.e., greenhouse, farmland with film mulching, and farmland without film mulching) and microplastics (MPs) might influence pesticide residues in soil. This study revealed an association between MPs and NEOs in realistic agricultural settings. Random forest models offered more reliable predictions than linear models and indicated that MPs, farmland type, and total nitrogen are key contributors to NEO levels.

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3.03.P-Mo199 Analysis of the Contamination and Reduction of Pesticide Residues During Transition from Conventional to Organic Rice Production in the Mekong Delta of Vietnam

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Rice cultivation in the Mekong Delta of Vietnam plays a critical role in both food security and the national economy. However, intensive agricultural practices, including the widespread use of pesticides and fertilizers, have raised concerns regarding environmental sustainability and human health. This study aims to evaluate pesticide residues in the Mekong Delta, with a focus on the three provinces Vinh Long, An Giang and Dong Thap, and assess the feasibility of transitioning from conventional to organic rice farming practices. Household surveys were conducted in the three provinces to understand farmers' pesticide usage patterns. Therefore, the farmers were asked for information about which pest dominates on their farm, which pesticides are being used, on the frequency of the pesticide usage, and their application dose. Additional information about major crop, crop rotation, number of harvest per year, and farm size were also provided. The surveys revealed a diverse array of 85 different pesticide brands corresponding to 51 active ingredients, with insecticides comprising the majority, followed by fungicides and herbicides. Additionally, soil and water samples were collected for pesticide residue analysis in rice fields in each province respectively. Pesticide residues were extracted using solid-phase extraction for water samples and accelerated solvent extraction for soil samples. For the analysis of pesticides a non-target screening was carried out using LC-MS/MS and the Quanpedia data base, which contains the information of 750 widely used pesticides. The non-target screening identified 74 active compounds belonging to the categories Fungicides, Herbicides, Acaricides, Insecticides. Moving forward, ongoing farmers survey s will help to assess regional variations in pesticide usage, while targeted quantification of select compounds will provide insights into contamination levels. Ultimately, this study contributes to our understanding of pesticide pollution in rice farming regions and informs strategies for promoting sustainable agriculture and safeguarding environmental and human health in the Mekong Delta.

3.03.P-Mo200 Pesticides Contamination in the Irrigation System of Arequipa, Peru

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Agriculture is the second-largest economic activity in Peru, playing a significant role in the country's exports. Yet, agricultural practices in the country rely heavily on using pesticides and other chemicals to enhance and secure food production, raising public health and environmental concerns. Therefore, in this study, we aimed to detect the presence of various pesticides in the irrigation system of Arequipa, Peru. A

sampling campaign was conducted in May 2022, analyzing water samples from key areas, including the Chili River, water ponds, and the Aguada Blanca and El Pañe reservoirs. Pesticide analysis involved water filtration, solid-phase extraction (C18 SPE cartridges), and a quantitative LC-MS/MS workflow. A total of 15 pesticides were analyzed in 26 samples. Results revealed the presence of 7 pesticides not approved under European regulations, although none of them are restricted by Peruvian regulation. Furthermore, Peruvian water quality standards do not include the monitoring of these pesticides, highlighting regulatory gaps. The detected pesticides included diuron, tebuconazole, propiconazole, carbendazim, azoxystrobin, and imidacloprid, confirming contamination of irrigation water bodies. This contamination suggests the existence of risks to farming workers, to consumers and to the ecosystems directly associated with the irrigation system. Further ongoing work is applying suspect screening analysis to a larger number of pesticides and other organic contaminants. The findings denote the need for stronger pesticide monitoring and regulatory measures in Peru and serve as critical evidence for governmental institutions to improve risk management and environmental protection policies.

3.03.P Measuring, Modelling and Monitoring the Environmental Behaviour and Exposure of Pesticides

3.03.P-Mo171 Sorption-Mediated Bioavailability and AMPA Influence on Bacterial Glyphosate Transformation

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Glyphosate (N-Phosphonomethyl-glycine)- the most widely used herbicide globally - and its transformation product, aminomethylphosphonic acid (AMPA), frequently coexist in various environments. While the biotransformation of glyphosate has been well-demonstrated under phosphorus (P)-limiting conditions, the ubiquity of other organophosphorus compounds that can also serve as P-sources raises questions about its actual biodegradation potential in real-world scenarios. This study investigated the biodegradability of glyphosate in batch cultivation experiments, focusing on the co-presence of AMPA, with or without a sorbent mineral. Experiments were conducted using the bacterial strain *Achromobacter insolitus* Kg 19 (VKM B-3295) that is able to utilize both glyphosate and AMPA as P-sources. The influence of goethite, a widely distributed iron oxyhydroxide in the environment, was evaluated for its potential to restrict biotransformation by sorbing glyphosate and/or AMPA. Preliminary findings indicated that glyphosate and AMPA exhibited a strong affinity towards goethite, influencing their bioavailability and subsequently the observed transformation kinetics. Additionally, in the absence of sorbent, AMPA was a more preferred P-source compared to glyphosate for the strain A. Kg 19. These results provide valuable insights into the environmental persistence of glyphosate in the presence of AMPA while emphasizing the need to consider the interplay between sorption and biotransformation a factor that requires further systematic investigations. Given the widespread occurrence of AMPA and other OP compounds, our findings suggest that their presence may hinder glyphosate biotransformation, potentially leading to lower transformation rates than those indicated by existing literature.

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3.03.P-Mo172 Effect of Soil Particle Size and Organic Matter on Pesticide Sorption Behavior in Agricultural Soils

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Intensive agricultural practices heavily rely on land exploitation and high inputs of pesticides to produce crop monocultures. Some applied compounds persist in soil, whereas the more mobile pesticides run off or leach through the soil profile and enter surface and groundwater sources. While the overarching mechanisms of pesticide sorption to soil constituents are acknowledged, there remains a lack of understanding regarding the relative contributions of specific soil size fractions and their associated organic matter.

To address this gap, and to discern how the physical characteristics of the soil fractions and their organic matter content contribute to pesticide retention, our research investigates the differences in pesticide sorption between coarse and fine soil size fractions with and without organic matter. Using wet sieving,

we first fractionate the soil into the coarse ($> 53 \mu\text{m}$) and fine ($< 53 \mu\text{m}$) soil physical fractions. After removing organic matter from soil size fractions, we obtain the coarse and fine mineral fractions. We perform batch sorption tests to determine the efficiency of 12 pesticide removal from soil solution using separated and treated soil fractions. Our approach allows a comparison between the effects of organic matter and mineral fractions, providing a clearer understanding of the contributions of each component to the overall sorption process.

Our hypotheses are: (1) separated soil fractions with intact organic matter will perform better in pesticide sorption than mineral fractions; (2) the extent of pesticide sorption efficiency will be different between the coarse and fine soil fractions; (3) we will be able to identify patterns regarding the most efficient fractions for the studied compound sorption. The findings of this research provide new insight into the effect of soil properties in reducing pesticide runoff, leaching, and subsequent environmental contamination and thus have important implications for choosing agricultural land-management practices (tillage, animal manure application, etc.).

3.03.P-Mo173 UAV Spray Applications as Innovative Solution for Heroic Vineyards: A Case Study in Northern Italy

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In Italy, several vineyards are in hills and mountains, and their management is mainly conducted through manual operations. The European Commission is hypothesizing to allow aerial products application in critical vineyards to manage diseases. This work aims to determine if UAV spray may represent an alternative to manual spray in this context.

During season 2024 a UAV spray system, fixed to the ground, was compared to a ground spray for grapevine downy mildew control during different three phenological stages. The trials were conducted in two vineyards, located in Grugliasco (TO) and Piacenza (PC) (Italy), comparing two commercial products, a contact copper-based fungicide (CU) and a systemic (metalaxyl-m) fungicide (MET), both sprayed from the ground (CU and MET) and from above canopy (UAV Cu and UAV MET). Leaves were collected from the vineyards after treatment at fixed intervals and artificially inoculated in the laboratory with a suspension at 5×10^4 of *P.viticola*, the causal agent of grapevine downy mildew. Then leaves were incubated at 20°C until symptoms were visible. Disease control was evaluated in terms of fungicide efficacy (%). At the same fixed intervals from fungicide application, other leaves were collected, about 20 g of fresh leaves, and used for the analytical determination of the products deposited on leaves.

An analysis of variance (ANOVA) was performed on efficacy data, post hoc Tuckey test was conducted to separate means ($p = 0.05$).

Based on the first trial results, ground spray copper-based and metalaxyl-m fungicides showed the highest efficacy, above 85% in Torino vineyard, not significantly different from UAV MET efficacy (about 70%), but significantly higher ($p = 0.04$) than the efficacy of copper-based fungicide from aerial spray (about 40%). In Piacenza vineyard, the efficacy was generally lower than Torino, between 50 and 65%, without significant differences among treatments, even if UAV CU presented the lowest efficacy. Even if aerial spray may have possible issues in covering the canopy, as found for the copper efficacy from aerial spray. UAV spray system seems to provide good disease control.

Two additional trials are running to evaluate the fungicide application during different phenological stages and different canopy heights and to optimize product distribution.

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3.03.P-Mo174 Environmental Parameter-Calibrated Models for Predicting Sediment-Water Partitioning Behaviors of Diverse Emerging Contaminants in Lotic Waterways

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The partitioning behavior of organic contaminants is critical to their mobility, bioavailability, and ecological risk in aquatic environment. However, developing robust models for predicting sediment-water partition coefficients (K_{oc}) in field waterways is challenging due to complex impacts of environmental factors on partitioning behavior. Here, we developed environmental parameter-calibrated models to predict the K_{oc} values of 10 classes of emerging contaminants (log K_{ow} ranging from -1.8 to 10.2) in the Guangzhou reach of the Pearl River, South China. Traditional models based on physiochemical properties

poorly predicted the field measured Koc values. By calibrating using climate change related environmental parameters, i.e., temperature, dissolved oxygen, pH, conductivity, and salinity, the prediction accuracy pronouncedly improved from 22% to 81%. This calibration effectively reduced the impact of season variations on Koc predictions, except for herbicides in both seasons and polycyclic aromatic hydrocarbons in wet season, suggesting that additional parameters are needed for these contaminants. Temperature was characterized as the principal factor influencing Koc predictions, except for per- and polyfluoroalkyl substances (PFASs) and neonicotinoids, which were mostly affected by pH. Salinity also exhibited significant effects on PFASs and herbicides. With measured water concentrations and water quality parameters, the developed model was validated to successfully predict sediment concentrations in lotic waterways. These findings provide insights into the effects of environmental factors on distribution of organic contaminants in highly dynamic waterways, aiding in predicting their fate and risk in aquatic ecosystems under the influence of climate change.

3.03.P-Mo175 Effect of Fertilizers on Dissipation Kinetics of Imidacloprid in Cultivated and Non-Cultivated Arid Soils

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Imidacloprid (IMI) is a systemic pesticide, that is widely used in plant protection around the world. After its application, several factors affect IMI degradation, such as soil characteristics and agricultural practices. This study explores the impact of fertilizer types on IMI dissipation in cultivated soil (CS) and non-cultivated soil (NCS) from Saudi Arabia. Four fertilizer treatments were used: three inorganic fertilizers (urea, monoammonium phosphate (MAP), and diammonium phosphate (DAP)), and one organic fertilizer (compost) were applied to long-term CS and NCS, followed by IMI addition and a 45-day incubation period. The results indicate that IMI degrades notably faster in CS treated with compost, exhibiting a half-life of 14.3 days compared with 20.1 days in NCS with the same treatment. For other treatments, CS half-lives ranged from 25.0 to 35.5 days, while NCS half-lives ranged from 34.7 to 40.5 days. For the controls fortified with IMI but no fertilizer, half-lives of 39.1 and 43.0 days were observed for CS and NCS, respectively. Both compost and urea addition accelerated dissipation, possibly due to enhanced microbial activity and growth post-treatment. Conversely, NCS exhibited slower dissipation attributed to lower organic matter and nitrogen content. Compost enhances IMI degradation, especially for nutrient-deficient soils; thus, the temporal efficacy of IMI will vary by soil and amendment types. Future research should investigate the synergistic effects of organic and inorganic fertilizers in facilitating the breakdown of IMI residues across soils from different origins. With further research, specific metrics for the application of IMI to meet the aim of pesticide application without excess pesticide residue could be developed.

3.03.P-Mo176 A Simplified Approach to Represent Bioturbation Effects on Pesticide Contents in Upper Sediment by the TOXSWA Model

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Bioturbation is recognized as a critical process influencing pesticide transport within upper sediment layers. Traditional pesticide fate models often represent this as a diffusive process, with the biodiffusion coefficient (D_b) serving as a key parameter. However, obtaining accurate values for D_b can be challenging, especially when limited information about benthic organisms is available.

This study introduces a fast and simplified approach to represent bioturbation effects on pesticide contents in sediment layers. The approach is specifically tailored for mobile compounds, i.e., with a low adsorption capacity. We calculate an effective diffusion coefficient as the sum of molecular diffusion (accounting for tortuosity) and a faunal activity diffusion component (independent of tortuosity). The faunal activity diffusion can be calibrated using experimental data, and the resulting calibrated value can be compared to literature on compounds primarily present in pore water, to assess whether bioturbation is indeed the primary process involved or if other processes may better explain the observed transport.

We implemented this approach within the TOXSWA model, which simulates pesticide fate and transport in small surface water systems. This adapted TOXSWA version will be applied to simulate linuron penetration in sediment of two experimental ditches in Renkum, the Netherlands and results will be presented on the poster. Earlier simulations suggested the occurrence of additional transport processes, next to molecular diffusion and sorption.

The implemented approach is designed for mobile substances, as it focuses on pore water concentrations

without addressing sediment reworking (with attached sorbed substance). While initially motivated by observations of linuron in experimental ditches, future work could extend the approach to sediment transport processes. The study emphasizes the importance of including additional processes, such as bioturbation, in regulatory frameworks, where they are currently not considered. The proposed TOXSWA version offers a practical way to investigate such processes and their implications for pesticide exposure assessments.

3.03.P-Mo177 Global Meta-Analysis and Machine Learning Reveal the Critical Role of Soil Properties in Influencing Biochar-Pesticide Interactions

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Biochar application is widely promoted for its dual benefits of boosting agricultural productivity and sequestering carbon. However, concerns remain about its environmental impact, especially regarding interactions with pesticide residues in soil. Previous research has shown elevated pesticide residues and prolonged persistence in biochar-amended soils, suggesting a potential negative impact on pesticide degradation, though conclusive evidence is lacking. To address this, a comprehensive assessment combining meta-analysis and machine learning was conducted, synthesizing data from 58 studies and 386 observations across field, pot, and incubation experiments worldwide. Contrary to initial concerns, our findings revealed no definitive increase in soil pesticide concentrations with biochar use. Additionally, pesticide concentrations in soil organisms, such as plants and earthworms, were reduced by 66%. Our research stands out for its thorough consideration of the variability in soil, pesticide, and biochar properties when assessing biochar application. Soil properties were identified as critical factors governing the fate of pesticides affected by biochar application. Additionally, we explored how different types of biochar and pesticides may exert varying effects. The quantitative analysis emphasized three key aspects in understanding biochar-pesticide interactions: (1) Soil Properties: The impact of biochar on pesticide residues is significantly influenced by soil properties such as soil organic matter (SOM) and pH. Soils rich in SOM were less likely to show increased pesticide persistence with biochar, while low-SOM soils often exhibited higher pesticide retention, raising potential risk. (2) Pesticide Properties: Biochar's impact on pesticide behavior varies with the physicochemical properties of the pesticides. Triazole and organofluoride pesticides showed increased persistence, potentially raising risks, while triazine and organophosphorus pesticides exhibited reduced persistence. (3) Biochar Properties: Wood-derived biochars generally showed stronger pesticide adsorption, underscoring the role of biochar composition in modulating pesticide persistence. By investigating the interactions between biochar and pesticides across varying soil conditions, this study provides a valuable reference for developing risk-assessed and informed strategies for biochar application.

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3.03.P-Mo178 SWAN's Evolution: Bridging Recent Modelling Advances and Regulatory Risk Assessment

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SWAN (Surface Water Assessment eNabler), sponsored by CropLife Europe (CLE) and developed by Hybrid Intelligence (formerly Tessella), has been a cornerstone in surface water regulatory risk assessment modelling since 2006. This poster presents recent advancements in SWAN, highlighting CLE's commitment to computational excellence and user-centric improvements.

SWAN provides a user-friendly interface, allowing the user to reliably apply modifications to STEP 3 runs, reflecting mitigation measures (drift-reducing nozzles, vegetative buffer strips, no-spray buffer zones), to subsequently run scenarios integrating these mitigations at STEP 4. SWAN itself does not perform the calculations but acts as an intermediary by modifying existing STEP 3 run files in a transparent and consistent manner. Every new SWAN release is validated and all processes are fully documented; SWAN efficiently automates the calculations but is reproducible manually by advanced modellers.

VFSMOD, a tool for simulating pesticide runoff mitigation through vegetative filter strips, has been an option in SWAN since 2012. VFSMOD has undergone various mechanistic upgrades in recent years including a mechanistic pesticide trapping, a dynamic predictor for runoff sediment inputs based on site conditions, and a new soil leaching and surface residue remobilization algorithm. To better integrate these advances into regulatory risk assessments, new vegetative filter strip scenarios were developed for FOCUS scenarios R1-R4.

With the expansion in regulatory interest of this increasingly sophisticated risk analysis tool, it is essential to balance complexity with sound software engineering and transparency. Previously, SWAN interfaced with VFSMOD through an external wrapper. In SWAN 5.2, the wrapper has been fully integrated into SWAN's core codebase, marking a significant improvement in the software's architecture. In addition to a gain in performance (typically 90% or greater reduction in runtime across our test scenarios), the incorporation also improves testability and transparent reporting, with the aim to maintain confidence in the tool's compliance with regulatory requirements.

The upcoming SW Repair version of SWASH will be coupled with a concurrent release of a new FOCUS-SWAN, ensuring closer alignment with SWASH release cycles and consistent compatibility.

3.03.P-Mo179 Derivation of Updated SWAN-VFSMOD Scenarios for FOCUS Step4 Simulations

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The SWAN tool for higher-tier surface water exposure assessment implements runoff and erosion reduction efficiencies according to the report of the FOCUS Landscape and Mitigation Working Group. Since 2012 SWAN also includes the option to apply mitigation using the mechanistic, dynamic and event-based vegetative filter strip (VFS) model VFSMOD. VFSMOD simulations had been performed to develop realistic worst case VFS scenarios for the four FOCUS runoff scenarios, which were subsequently included in SWAN. The current version of SWAN (v.5.0.1) still uses the same VFSMOD version as in 2012. However, since then substantial scientific developments have been made to further improve VFSMOD and its underlying assumptions and process descriptions. Moreover, the population of the spatial cumulative distribution functions (CDFs) needed updating. The objective of this study was therefore the corresponding update and improvement of the SWAN-VFSMOD scenarios for EU pesticide risk assessment.

The FOCUS scenario shapefiles were intersected with CORINE Land Cover and subsequently linked with the SPADE2 and SPADE14 soil profile databases. The valid 1897 scenario/profile combinations were simulated with VFSMOD. First, two rainfall/runoff events (duration 1 h and 8 h) for each R scenario were created with the tool UH v.3.07. VFSMOD was run for the above events, two example compounds (Koc = 100 L kg⁻¹ and 10000 L kg⁻¹), a VFS length of 10 m, and two different variants: A) profiles with hard rock in < 100 cm depth were simulated with the shallow water table option; B) all profiles were simulated with classical Green-Ampt infiltration.

In a next step, area fractions per Soil Mapping Unit were calculated for each scenario/profile combination. These area fractions were subsequently used to calculate spatial CDFs of the target variable ?P (relative reduction of pesticide load by the VFS). Finally, from each CDF the profile corresponding to the 10th spatial percentile of ?P was extracted. From the created CDFs the following combination was selected for the new SWAN-VFSMOD scenarios: Koc = 100 L kg⁻¹, duration = 8 h, and variant B.

A set of updated 90th percentile worst case VFSMOD scenarios for FOCUS step4 simulations has been derived. The updated VFSMOD scenarios are both more realistic and more conservative than the old scenarios (a comparison of ?P will be presented) and are ready to be integrated in SWAN 5.2. A technical report and a peer-reviewed publication are in preparation.

3.03.P-Mo180 Modelling Framework to Identify FOCUS Step 3 Surface Water 'Screening' Scenarios Following Use of REACH-IN LET

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Substances used as co-formulants in plant protection products (PPP) must be registered in the European Union under the REACH Regulation (EC) No 1907/2006. The standard environmental exposure assessment for chemicals under REACH uses a multicompartamental mass-balanced model and, at the local-scale, is based on urban (wide dispersive) or industrial (point source) emissions. However, environmental releases of co-formulants used in PPP to agricultural soil and indirectly to waterbodies adjacent to treated fields are not in scope of this assessment framework. The surface water module of CropLife Europe Local Environment Tool (LET) was developed to assess local environmental exposure to

co-formulants in surface water and sediment based on the standard FOCUS Step 2 model used for PPP. It combines the local PECs with regional PECs resulting from emissions of other nonagricultural uses of the same substance, to perform risk characterizations on the local scale which conform to REACH requirements.

To facilitate refined assessment of surface water exposure to co-formulants, a framework has been developed to identify FOCUS Step 3 screening scenarios based on three substance sorption (KOC) categories: KOC = 100 L/kg, KOC = 1000 L/kg, and KOC = 10000 L/kg; and three biodegradability categories: Readily Biodegradable, Inherently Biodegradable, and Not Biodegradable. Application methods (i.e., ground spray and air blast), application timings covering each month of the year, and representative crops including pome fruit, fruiting vegetables, winter cereals, potatoes, and field beans were assessed. The application schemes were developed into a simple framework allowing selection of reasonable worst-case scenarios for surface water assessment of co-formulants at FOCUS Step 3. For co-formulants with $100 < \text{KOC} < 1000$ L/kg, spray drift was the main exposure route for crops with air blast application, whereas drainage or runoff was the major route of exposure for crops with ground spray application. The proposed scheme for these co-formulants is to select application timing from the product GAP or in case of a wide application window, application timing dependent on KOC category. For readily or inherently biodegradable co-formulants with $\text{KOC} > 1000 < 10000$ L/kg, spray drift was the major exposure route for all crops and application methods assessed. The proposed scheme for these co-formulants is to select application timing from the product GAP.

3.03.P-Mo181 Reduction of Complexity: Variance-based Sensitivity Analysis for FOCUS STEP 3 *Dimitrios Alexander Skodras¹ and Judith Klein², (1)Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany, (2)Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany*

To standardize the role of models in the European Union (EU) plant protection product authorization process, the Forum for the Co-ordination of pesticide fate models and their use (FOCUS) has established guidelines, including the FOCUS STEPS models for surface water risk assessment. Conservative scenarios representing EU member states are used, with a 4-tier approach to assess limit value exceedances. While higher-tier calculations provide greater accuracy by requiring more information, they do not incorporate the uncertainty of model parameters, potentially leading to significant inaccuracies if sensitive parameters.

The project employs sensitivity analysis using the Sobol' method to identify influential parameters in FOCUS STEPS models. Model parameters are sampled using low-discrepancy sequences, and Sobol' indices are calculated to determine the sensitivity of the FOCUS STEPS models to parameter variances. The analysis centers on the first order Sobol' indices representing the main effects, providing insights into parameter influence. Higher order indices are examined too.

FOCUS STEP 3 incorporates the TOXSWA model, which calculates pesticide concentrations in surface water by receiving input on pesticide fluxes from drift and runoff (calculated with PRZM) or drainage (calculated with MACRO). These simulations are run for three types of surface waters across 10 representative scenarios encompassing the meteorological and soil diversity in Europe.

For the sensitivity analysis, both substance and scenario parameters are included to explore regions in parameter phase space where the models show high sensitivity to certain parameters over others. A key purpose of this analysis is to flag highly sensitive scenario parameters, allowing for more precise information gathering and improved control over environmental variabilities and uncertainties.

Additionally, the project aims to simplify the user interface by highlighting sensitive substance properties for which the reduction of uncertainty would be beneficial and less sensitive properties for which a central value might be sufficient. Thus, guiding a more focused allocation of resources.

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3.03.P-Mo182 Probabilistic Spray Drift Model for Realistic Spray Applications in Arable Crops in the Netherlands

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Deposits of spray drift onto edge-of-field watercourses contribute significantly to the exposure to pesticides for aquatic organisms. For regulatory purposes, still the major approach in exposure assessment is to use average drift deposition curves with wind direction perpendicular to the field edge. Few years ago, in a more realistic approach, a probabilistic exposure model has been developed for downwind deposits of spray drift for spray application in fruit orchards in the whole of the Netherlands, involving

upward/sideways directed spray applications. Recently, this approach was applied to spray application for downward sprayed crops (arable crops). In principle, the IDEFICS spray drift model for arable crops is suitable to be used in this probabilistic method. However, the method typically involves 100,000 simulation runs for a single countrywide probability estimation, which are impossible to carry out using IDEFICS. Therefore, first a statistical metamodel was derived from IDEFICS, for an appropriate number of simulation runs with relevant parameter settings. The metamodel was calibrated to closely fit the WUR Drift Calculator (WDC) for parameter settings equivalent to those used in the WDC. Next, the metamodel was implemented in the newly developed countrywide probabilistic model, named PROSPEX. The latter model required as inputs the frequency distributions of meteorological factors (wind speed, wind direction, temperature, relative humidity) for different regions across the Netherlands. Distributions of edge-of-field watercourses next to arable fields across the Netherlands were required inputs as well. Crop related aspects were based on Ctgb's list of all crops grown in the Netherlands (DTG list). WDC's drift mitigation measures were implemented as well. In this way, the PROSPEX model can be considered as an extension of the WDC for more realistic simulations. Currently, it is being implemented in the DRAINBOW model, which combines different pesticide exposure routes and fate in edge-of-field watercourses for higher-tier regulatory use in the Netherlands.

3.03.P-Mo183 Assessing Connectivity of Uncertain Field Sites in Groundwater Tier-4 Programmes using Conservative Tracers

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Groundwater (GW) monitoring is the highest tier (Tier-4) in the leaching assessment of plant protection products (PPPs) in the FOCUS GW assessment scheme. To ensure GW monitoring sites are fit for purpose (i.e., to address vulnerable real-world worst-case scenarios), proving hydraulic connectivity between the field and sampling well may be necessary. GW monitoring sites are installed in real-world conditions and can face variability in hydrogeological settings, which can impact their use as vulnerable monitoring locations. To address this issue tracer tests can be performed on sites where GW flow assessment is variable or site design is questioned. By establishing the degree of hydrogeological connectivity between treated fields and wells, the accuracy of leaching estimates in groundwater risk assessment can be supported.

In this case study four sites with uncertain connectivity in Italy were selected. These sites are part of an ongoing GLP GW monitoring programme (edge-of-field setting), providing realistic and quantitative information for Tier-4 monitoring programmes. A common tracer, Potassium Bromide (KBr), was applied to sub-plots at each site. GW samples were collected from three or four wells installed around the field edge. At each site GW samples were collected regularly (weekly to quarterly) over one year to track the movement of bromide ions in the subsurface. At each site information on site characterization (e.g., depth to water, weather and rainfall) and other ancillary data (e.g., ditch status and rivers) was also collected. Ion chromatography was used to analyse for Bromide.

Initial results suggest that three out of four sites have wells hydrologically connected to the surface of the field. The fourth site shows a complex behaviour, being influenced by nearby surface features, and is still under investigation. This work shows how to design and perform a connectivity assessment at hydrogeological sites for a Tier-4 GW monitoring study aimed at evaluating the potential leaching of PPPs. By addressing uncertainties at the field scale, trust in the employment of Tier-4 data in plant protection product risk assessment will contribute to informed decision-making processes of PPPs.

3.03.P-Mo184 Groundwater Dilution Factor Evaluation in the EU Using the Spatial LUCAS PEARL Modelling Framework

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In 2023, EFSA and ECHA have published a new guidance on the impact of water treatment of Plant Protection Products (PPPs) residues for drinking water. The guidance assumes that PEC_{gw} at 1m soil depth (Tier 1/2/3 of FOCUS GW) will be the final exposure indicator for evaluation, without considering any dilution factor (DF) across different EU regulatory zones. The attenuation of PPP concentrations along the flow path from the regulatory PEC_{gw}, 1m to the potential raw groundwater abstraction location has been scientifically discussed on various scales through either data analysis or modelling assessment. However, there is currently no systematic study available at the EU level for estimating groundwater DFs. Furthermore, the recent EFSA statement on groundwater monitoring and exposure assessment from 2023 suggests that a depth of 1.2m below the shallow groundwater table (i.e., GWT < 10 m) can be used as a conservatively representative depth for aquifer quality evaluation. In light of this, we have developed a spatial modelling framework to investigate the EU-level groundwater DFs from PEC_{gw}, 1m to PEC in the 2m thickness aquifer below GWT (PEC_{gw}, 2m-aquifer).

Over 5,000 virtual well catchments (VWCs) were derived using LUCAS 2018 soil points as pour points for drainage area calculation, utilizing the EU digital elevation model through hydrological assessment. In the soil unsaturated zone of each VWC, the LUCAS soil data was implemented in the 1D leaching model FOCUS-PEARL 5.5.5 for calculating the maximum PEC_{gw,1m} over a 100-year simulation period, as well as the total leaching mass over entire agriculture land to aquifer at the year when maximum PEC_{gw,GWT} occurs. In the aquifer of each VWC, a simple well-mixing reservoir model was employed to calculate the PEC_{gw,2m-aquifer}, considering spatially distributed porosity in deriving aquifer storage volume. Nine dummy substances scenarios with varying DT₅₀ values ranging from 10 to 200 d and K_{f,oc} values from 10 500 mL/g were used for quantifying DFs by equation of maxPEC_{gw,1m}/PEC_{gw,2m-aquifer} for each VWC. Our calculated 50th percentile DF reached 26 (n = 12,199) in the Northern Zone, 87 (n = 25,413) in the Central Zone, and 117 (n = 21,866) in the Southern Zone. Our findings indicate that using PEC_{gw,1m} as the final exposure indicator for drinking water abstraction evaluation is overly conservative even under the EFSA recommended representative groundwater depth for shallow aquifer.

3.03.P-Mo185 Enhancing Groundwater Exposure Assessments for Pesticides: Addressing the Challenges of Monitoring Studies in the Absence of Harmonized Guidance Document

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Groundwater monitoring represents a higher-tier option in the groundwater exposure assessment of Plant Protection Products (PPPs) in the context of the peer review process for evaluating active substances and their metabolites in the EU. However, a harmonized guidance document on how to conduct such monitoring and interpret the results is missing. A scientific paper on the design and conduct of groundwater monitoring studies supporting pesticide groundwater exposure assessment was published by Gimsing et al. (2019). This paper set the basis for developing a guidance document on pesticides groundwater monitoring. At the request of the European Commission, EFSA PPR Panel reviewed the paper by Gimsing et al. (2019) and wrote a statement (EFSA, 2023).

According to the EFSA statement well-conducted monitoring studies provide more realistic exposure assessments, potentially superseding findings from lower-tier studies. However, the lack of Exposure Assessment Goals (ExAG) hampers the development of an harmonized guidance on designing, conducting, and evaluating monitoring studies for regulatory purposes. In the absence of a specific guidance document, the evaluation and use of submitted monitoring studies during the peer review is challenging.

This study is based on the most recent discussions from expert meetings held during the peer review process, following the publication of the EFSA statement. These meetings focused on the evaluation of monitoring data and their acceptance for higher-tier groundwater risk assessments. Unresolved issues on study design were addressed, including uncertainties on monitoring depth, site selection, groundwater flow direction variability, and application patterns. Emphasis was put on the need for harmonized models and guidelines for vulnerability assessments to support site selection and context setting. A positive outcome was the agreement on how to present result tables. However, no final agreement was reached on a specific type of regulatory endpoint, and it was concluded that the use of the 90th (spatial/temporal) percentile concentration is not defensible for groundwater monitoring data. A higher percentile might provide a more robust regulatory outcome. Finally, it was stressed that the absence of ExAG for groundwater leaching at the EU level remains a significant limitation. Without clear ExAG, it is difficult to establish standardized procedures and evaluate monitoring studies consistently for regulatory purposes.

3.03.P-Mo186 Challenges for Soil Photolysis Modelling in Groundwater Risk Assessment: An Analysis of Surface Layer Dynamics in FOCUS Models

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The European Food Safety Authority (EFSA) guidance on soil phototransformation products in groundwater (GW) recently mandated the use of a soil photolysis module within FOCUS PELMO. Initial tests indicate that the extent of photolysis in FOCUS PELMO simulations is highly sensitive to environmental processes governing the duration for which chemical mass is retained within the photolytically active topmost one millimeter of soil. Notably, this guidance was implemented despite EFSA's acknowledgment that FOCUS GW models were not developed nor calibrated to accurately predict chemical fate and environmental processes in the surface layer.

This study analyses water, soil, and compound behaviour within the surface layers of FOCUS PELMO and compares it to the predicted behaviour in FOCUS PEARL. As the photolysis module has not been implemented in FOCUS PEARL, precluding direct comparison of photodegradation between the models, we assess models' susceptibility to photolysis by analysing the residence time of substances in the surface

layer, i.e., the exposure to photodegradation. In the absence of true validation for either model against environmental data in the topmost layer of soil, this analysis provides model cross validation. A direct comparison of the two models reveals that while soil temperature correlates strongly ($R = 0.85-0.94$), soil moisture ($R^2 = 0.07-0.33$) and water fluxes ($R^2 = 0.33-0.83$) differ substantially. The hydrological disparities between the models result in differences in substance residence time in the soil surface, significantly impacting susceptibility to photolysis. We conclude that implementing a photolysis module within PEARL would likely yield PECs that differ significantly from those produced by FOCUS PELMO. Thus, both models will require calibration at the topmost soil layer similar to that performed at 1m depth. This therefore raises concern regarding the reliability of risk assessment decisions based on the current in force guidance.

3.03.P-Mo187 Modelling bromide breakthrough in railway lysimeters

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Lysimeters exposed to natural precipitation were used to study the leaching behavior of various herbicides in railway track soils. We focus here on the leaching dynamics of the commonly employed conservative tracer bromide, aiming at characterizing the lysimeters in terms of effective transport volume and dispersion of solutes.

Once a year, we applied bromide to some of our ten field lysimeters (0.5 m² x 1.45 m), which are filled with railway materials from three different locations (i.e., there are two triplicates and one quadruplicate) and analysed bromide in the leachate. Breakthrough curves (BTCs) were determined for up to five years for one and the same lysimeter, allowing us to link the BTCs to the meteorological conditions (i.e., inter-year comparisons). As data for each lysimeter are available, we can also investigate the influence of the type of the soil on the BTCs as well as differences between lysimeters filled with the same material (i.e., inter-lysimeters comparisons).

We used an analytical model to fit the bromide BTCs, assuming steady-state flow and a constant water content over depth in a homogeneous soil. The model fitted relatively well to the experimental BTCs, with calculated bromide recoveries ranging from 74% to 103%, compared to measured values of 55% to 105%. Dispersivity, a measure of how substances spread through the soil column, varied both within and between the three lysimeter materials, as well as across years. Notably, higher stone content was associated with an increased dispersivity (i.e., a lower amplitude and an extended duration of the BTC), but also an earlier onset of the BTCs. We also note that, within the same lysimeter, the effective volumetric water content that participates in bromide transport was higher during a wet year, suggesting that weather conditions play a significant role in how bromide is transported through the soil column.

Being able to model how bromide behaves in the different railway soils will greatly facilitate the interpretation of the leaching behavior of the herbicides applied to these lysimeters and their metabolites. Ultimately, the results of the study will assist railway companies in making more judicious decisions about the potential leaching risks of applied herbicides to groundwater.

3.03.P-Mo188 Assessment of Organochlorine Pesticides and Chlorpyrifos in Groundwater in the Yucatan Peninsula

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Yucatan Peninsula (YP) is formed by a large limestone platform with dissolution conduits and fractures that result in a complex coastal karst system that is highly permeable and therefore vulnerable to contamination. This system transports large volumes of groundwater with significant discharge in coastal zones, representing the only source of drinking water for the population. Among the anthropogenic activities carried out in the area, agriculture has a high water consumption rate and is associated with contamination by pesticides. Despite being restricted/prohibited in Mexico, there are still reports of the use of organochlorine pesticides (OCP) in the region. Published research reports high concentrations of these compounds in groundwater (supply wells and in the ring of cenotes). OCP are highly persistent in aquatic environments and have been detected in different environmental matrices in the YP: coastal and groundwater, beach and mangrove sediments, and protected organisms such as the green turtle. The aim of this research was to assess the presence of 19 individual OCPs and the organophosphate pesticide chlorpyrifos in 16 sites (15 monitoring wells and one cenote) in the northern YP, including the states of Campeche, Yucatán and Q. Roo. Water samples (n = 29) were collected in May 2024 and spatial geographic variation and two depths (superficial and middle depth) were considered. OCP and

chlorpyrifos were determined by solid phase extraction (SPE) and gas chromatography coupled to mass spectrometry (GC-MS) in single ion monitoring (SIM) mode. Despite being a non-restricted and frequently used pesticide, chlorpyrifos was not present in the water samples; in contrast, 14 individual OCPs were detected, all below the maximum permitted limits set by the Mexican standard for drinking water. The average concentration of total pesticides was highest in the wells of Q.Roo with 127.6 ± 42.8 ng/L, while the mean levels in the wells of Yucatan and Campeche were very similar, 54.0 ± 63.9 and 53.9 ± 57.4 ng/L, respectively. The most frequently detected pesticides were endosulfan I (81.3%) and DDE (75%). Endrin and endrin ketone were the compounds detected in the highest concentration with maximum values of 82.3 and 204.0 ng/L, respectively. A general tendency to detect higher levels in surface samples is observed. The presence of these restricted pesticides in the YP groundwater is a cause for concern, since their toxic effects have been widely described.

3.03.P-Mo189 CF3-Containing Pesticides and Widespread TFA Contamination of Groundwaters: Source Evidence and Uncertainties from Luxembourgish Studies

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Trifluoroacetate (TFA) is currently under focus because of the widespread pollution in surface and ground waters. TFA might jeopardize the total PFAS parameter threshold in drinking water (500 ng/L). Sources of TFA are manifold ranging from ubiquitous atmospheric input, over fluorinated pesticides, manure and fluorinated pharmaceuticals contained herein as well as potential industrial sources. While the latter are not relevant for drinking water protection zones in Luxembourg, the first three sources are. The initial approach was to monitor groundwater spring and wells with a gradient of nitrate pollution that represents variable agricultural land use in the capture zones. Nitrate values spanned from 4-77 mg/L and 40 sites were sampled along the gradient. Pearson correlation coefficient turned out to be 0.61 with the intercept at 522 ± 265 ng/L. Variability around the linear regression was substantial but residues showed normal distribution. Atmospheric wet deposition with 500 ng/L TFA concentrations have been reported throughout Europe. 28 of 40 samples exceeded 1000 ng/L with a maximum of 5630 ng/L. A projection of average CF3 containing pesticide use statistics for Luxembourg with the assumption of 100% TFA generation showed that the regression supported pesticides, especially the largely used flufenacet as a main source. However, simultaneously run analysis of flufenacet-ESA in the samples showed no detections with an LOQ of 5 ng/L. On the other side flufenacet-ESA is regularly detected in flood waves in surface waters, as is dimethenamid-ESA, whose parent compound dimethenamid-P has been also increasingly used in the recent years. Dimethenamid-ESA is being increasingly detected in Luxembourgish groundwaters and has shown a 33% positive detection in the set of 40 samples considered here. A possible explanation would be the selective degradation of flufenacet-ESA in the aquifer with residence times of 10-20 years. The poster will present the evidence, discuss uncertainties and open questions around the stoichiometry of Flufenacet- TFA degradation pathways.

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3.03.P-Mo190 Landscape Level Modelling for Derivation and Contextualization of Worst-Case Surface Water Dilution Factors at Drinking Water Abstraction Locations

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EFSA issued a guidance document on the impact of water treatment processes on residues of active substances or their metabolites in water abstracted for the production of drinking water. The guidance contains recommendations for the Step 1 screening process, the exposure assessment of plant protection products at drinking water (DW) abstraction locations, originating either from groundwater or surface water (SW). For drinking water abstracted from surface water, the guidance proposes calculating predicted environmental concentrations (PEC) at drinking water abstraction locations (PEC_{dw}) by applying dilution factors (DF) to edge-of-field PEC_{sw} calculated according to FOCUS (FORum for the Co-ordination of pesticide fate models and their Use). Worst-case dilution factors (DF) are to be used as the first tier; DF derived from GIS-based landscape-level assessments can be used as higher tier options. This study is an extension and continuation of a previous geospatial analysis that builds upon the Dutch DROPLET model and investigates generic dilution factors starting from edge-of-field PEC_{sw} and extending to a potential drinking water abstraction location downstream at the catchment outlet. This innovative approach incorporates agricultural land use and hydrology considerations, and utilizes a stepwise GIS-based approach at the EU level. The present study explores the significance of upstream agricultural land use, catchment hydrology, and connectivity of treated fields in relation to the proposed datasets and parameters outlined in the guidance, and beyond. Results show a significant difference when compared to the

proposed worst-case dilution factors outlined in the guidance. The methodology and datasets used contribute to a more realistic Step 1 screening process. To contextualize the proposed method, the Soil and Water Assessment Tool (SWAT) is utilized as a higher tier model. By integrating detailed parameters, SWAT provides a comprehensive framework for assessing water quality and quantity within a watershed in space and time. In this study, SWAT serves as a crucial tool for evaluating the dilution factors derived from the GIS-based analysis. SWAT also offers a higher level of detail, which enables a more nuanced understanding of the interactions between agricultural practices and water quality outcomes at a catchment level.

3.03.P-Mo191 Use of Geospatial Analysis Techniques to Evaluate Potential Risks of Plant Protection Products to Drinking Water Abstraction Points in the Netherlands

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In environmental risk assessment of plant protection products, geospatial analysis techniques can be used for modelling, contextualisation and vulnerability mapping, utilising surveys, monitoring data as well as satellite imagery. Spatially distributed modelling or use of monitoring data are considered higher tiers in the environmental risk assessments of active substances of plant protection products in the EU and Member States.

In this study, we applied advanced geospatial analysis techniques to evaluate the potential risks of a specific crop protection active substance to enter drinking water treatment systems (DWTP) in the Netherlands. Publicly available monitoring datasets, land use survey data, and web mapping services were combined to: (1) determine precise locations of water treatment plants, abstraction points, and water protection zones in the Dutch regions; (2) identify and map agricultural fields where the active substance was used; and (3) calculate the shortest distances between agricultural fields where the substance was used and the nearest surface water body and drinking water abstraction points.

Using Geographically Weighted Regression (GWR), we assessed how the relationship between crops cultivation and surface water concentrations of the active substance varied at the local scale around drinking water treatment plant (DWTP) abstraction points. The GWR method enabled us to capture spatial variation in the risk associated with surface water contamination more precisely, allowing for localized assessment that standard Dutch regulatory modelling approach using GeoPEARL, DROPLET or TOXSWA might overlook. Additionally, Hot Spot Analysis using the Getis-Ord G_i^* technique was employed to identify agricultural areas where potato cultivation posed a statistically significant risk to the drinking water abstraction points. These tools helped identify at-risk abstraction points with much greater accuracy, while also indicating that the nearest treated field was approximately 15 km from the DWTP. The combination of GWR and Hot Spot Analysis provided a robust workflow for the identification of spatial relationships between plant protection product uses and potential contamination of surface water abstracted for drinking water in the Netherlands. These geospatial analysis techniques could allow for more precise and informed higher tier risk assessments and regulatory decision-making in the Netherlands and other EU Member States.

3.03.P-Mo192 Scoping Refinements for Surface Water Metabolites in Relation to the Drinking Water Treatment Guidance

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The new Drinking Water Treatment guidance released by EFSA in July 2023 has led to many questions on how this will impact active substances and their metabolites in the European market. The guidance applies to both surface water and groundwater bodies, with all substances present at $\geq 0.1 \mu\text{g/L}$ considered relevant for assessment. While active substances tend to have a large amount of data available to refine the risk assessment, the same cannot be said for metabolites. In particular metabolites only observed in the water compartment often rely on default values. Therefore, a scoping assessment has been performed to assess if defined endpoints would improve the risk assessment and eliminate the need to assess these substances further.

Several substances with metabolites triggered under the Drinking Water Treatment guidance in surface water were selected. The options were assessed according to FOCUS surface water guidance using the default parameters and then a variation of possible DT50, KOC and formation fraction values that could be generated from laboratory studies. The results were then analysed to determine the impact of the refined endpoints of these metabolites for resolving the Drinking Water Treatment guidance requirements at Step 1.

3.03.P-Mo193 Muddy Waters. Navigating the EFSA/ECHA Drinking Water Treatment Guidance for Biocidal Products: Impacts, Challenges, and Uncertainties

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The EFSA/ECHA guidance document on the impact of water treatment processes on residues in water abstracted for drinking water production applies to both active substances and products under Regulation (EU) 528/2012 from 1st April 2026. Therefore, in many cases, the biocidal product application stage will represent the first time the active substance(s) involved will be evaluated against this guidance adding additional uncertainty to the registrability of biocidal products.

The guidance can be broadly divided into three parts: environmental exposure, water treatment transformation product (tTP) testing, human health hazard assessment. Understanding the nuances of the environmental exposure assessment is key as it triggers subsequent tTP testing and human health hazard assessment, which are expected to be costly and complex to conduct.

As of July 2024, there were 294 active substance-product type combinations approved in the EU. Using the publicly available data from the active substance approvals process, an assessment of the proportion of products likely to require tTP testing is made based on the environmental risk assessments conducted for the representative products. In considering the environmental exposure pathways and models across the different product types, uncertainties come to light in the application of the guidance and consideration of further impacts on the stewardship of biocidal products through the regulatory process is made.

3.03.P-Mo194 Robust and Representative Allocation of Arable Land in Spatial Layers used for Risk Assessments

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The concept of arable land is a cornerstone of any regulatory activity related to agriculture in Europe. However, the identification of arable land using geodata can be still challenging. The differences in sources, versions, resolutions and accuracies of geodata become evident in spatial analyses. This work aims to demonstrate challenges and questions that may arise in defining arable land within the regulatory context using Corine Land Cover (CLC). CLC repository includes 11 agriculture classes: three annual, three permanent uses, one pasture/grass, and four heterogeneous uses. In Europe, agriculture covers more than 2.1 million pixels (~211 million ha), representing 48.8% of the land use. Majority of agricultural land is non-irrigated arable land (56.9%), followed by pasture (17.1%), and complex cultivation patterns (10.2%). However, a variable subset of those classes is used to identify arable land. All classes excluding rice and pasture are proposed in the EFSA report on SW repair, covering 82.6% of all agriculture uses that include patchy and mixed classes (e.g., agro-forestry areas). Three annual crop classes, including rice, are instead used in the latest OM map of arable land, which reduces the bias of patchy classes but also decreases the coverage (only 58.6%). A middle ground would include complex cultivation patterns and annual crops associated with permanent crops but excluding rice, thereby reducing the bias given by the most patchy classes but increasing the coverage to 74.1% of agriculture uses. Though more recent versions are available, an outdated CLC2000 is included in the EFSA spatial dataset. Input data used in the risk assessment have not been updated for 24 years, thereby resulting in many uncertainties. For example, a resampled 1-km grid map is used instead of the original map: in heterogeneous areas, the class assigned to the cell may cover only ~25% of the land uses in the grid. In comparison, the soil parameters used in the risk assessment (e.g., pH, OM, BD) are not derived in the same way and have a different origin and resolution. Thus, soil parameters derived from geodata and used for risk assessment may have forest soil biases and may not be representative of agricultural uses. This risk is reduced in less patchy areas or where the CLC class is homogenous. This work indicates the need for a broader discussion around the use of geodata in risk assessment, particularly on robustness, transparency, and consistency.

3.03.P-Mo195 Global Pesticide Application Data as Input for Assessing Ecosystem Exposure and Impacts

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Agricultural pesticide applications significantly impact terrestrial and aquatic ecosystems, yet comprehensive data on actual field applications remains fragmented. We address this knowledge gap by developing the first global dataset of agricultural pesticide applications at country-crop-chemical level, incorporating detailed information on crop growth stages, application timing, and methods. This dataset aims to support the development of approaches that can predict how different application scenarios influence exposure and impact patterns across species and trophic levels in terrestrial and aquatic

ecosystems.

By synthesizing field-level application records from agricultural monitoring programs (2016-2020) and employing machine learning approaches including XGBoost and conformal prediction, we created a comprehensive dataset on pesticide application, including a spatially-explicit mapping at 5×5 arcminute resolution for each country-crop-chemical scenario. Our methodological framework integrated different datasets into a consistent format capturing application parameters, environmental conditions, and agricultural practices.

The resulting database encompasses more than 2.9 million tonnes of annual average pesticide use across 180 countries and 130 crops, involving 1077 distinct compounds. Four compounds dominate global usage: glyphosate (0.64 million tonnes), sulphur (0.17 million tonnes), mancozeb (0.16 million tonnes), and atrazine (0.1 million tonnes), with China, USA, and Brazil accounting for 46% of total mass application despite covering only 25% of global crop treated area. To address data gaps, we developed estimations for 81 nations, predominantly in sub-Saharan Africa, and supplemented missing application details for over 50% of country-crop-chemical combinations. The dataset provides compound-specific application patterns, including detailed timing and methods, exemplified by Acephate usage in US cotton and soybean production.

This comprehensive resource enables reconstruction of exposure scenarios, identification of chemical accumulation hotspots, and assessment of spatial and temporal exposure variations. The dataset uniquely supports development of targeted risk mitigation strategies and evidence-based policies for protecting biodiversity from chemical stressors, directly contributing to objectives such as the EU Zero Pollution Strategy and Biodiversity Strategy.

3.03.P-Mo196 Assessing Exposure to Pesticides with Spatially Distributed Environmental Scenarios at Field Level in Czechia

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Biotic, abiotic and agronomic parameters are the building blocks that constitute environmental scenarios providing context for exposure modelling to chemicals. Currently used exposure modelling frameworks, e.g., FOCUS surface water 2001, provide limited view on impact of spatiotemporal variability of environmental scenarios on estimated exposure profiles of agrochemicals in environment.

We compiled data related to application regimes of three Active Substances' (AS): glyphosate, tebuconazole and acetamiprid, followed by climate, topography, soil and hydrology data at individual field level using publicly available national, European and global datasets, such as, national crop maps and Plant Protection Products use statistics (PPP), PPP authorization registers, European Soil Data Centre (ESDAC) and Food and Agriculture Organisation (FAO). Data were then aggregated as Virtual Field across georeferenced agricultural fields in Czechia. Temporal (daily for year 2021) and spatial (whole-country individual fields) aspects of collected data, in particular georeferenced AS application patterns (GeMAPs), are at the centre of this work. The GeMAPs are obtained from the AS mass applied (g) and treated area (ha) for each AS-crop combination and then matched with georeferenced agricultural fields. PECsoil and PEC surface water (sw) were deterministically modelled. AS physico-chemical properties (KOC and DT50) are used to estimate initial and time averaged PECsoil for all fields in the country. Then, using daily precipitation monitoring data, daily generated surface runoff depth (mm) and AS dissolved fraction removed from topsoil are estimated for each field within a 100m buffer area around river segments. Finally, AS concentration in surface water is obtained using AS loads from adjacent fields and basin-specific river discharge volume. Application rates and estimated regulatory variables (PECsoil and PECsw) of the three ASs show significant spatial variability among individual fields not visible when the whole-district or coarser scales are considered pointing at possible pesticide exposure hot spots/moments. Data assimilation at the field scale opens the possibility for improving decision-making by locating exposure hot spots and hot moments and introducing amendment treatments. Further steps of this work will include integration of multi-annual crop data, daily precipitation, daily river flows and AS usage for prospective exposure assessment.

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3.03.P-Mo197 Challenge Accepted – First Ultra-Trace Level Monitoring Data of Pyrethroids in Bavarian Rivers

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Pyrethroids are widely used as insecticides, biocides and pharmaceuticals and cause adverse effects to aquatic ecosystems at very low concentrations. In addition, five pyrethroids are included in the draft of the amendment of Directive 2008/105/EC on environmental quality standards (EQS) with proposed EQS values down to the low pg/l-range. However, the detection of pyrethroids in this concentration range still represents a particular analytical challenge and thus only little monitoring data is available for adequate assessment of pyrethroids in surface waters.

Therefore, we developed a sensitive method for six common pyrethroids. Briefly the method consists of liquid-liquid extraction of 1 L surface water with 40 mL hexane and subsequent determination of pyrethroids with large-volume GC-EI-MS/MS. We reached limits of quantification (LOD) of 50 pg/l to 100 pg/l allowing a first assessment of proposed chronic EQS for most pyrethroids.

In 2022, pyrethroids were analyzed quarterly at ten major rivers across Bavaria. β -cyfluthrin and cypermethrin were not detected at all, while deltamethrin and esfenvalerate were found only very sporadically. Bifenthrin and permethrin were detected at five respectively six of the ten sampling sites with total detection frequencies of 30% and 33%. Only permethrin was determined at concentrations higher than 1000 pg/l, while the concentrations of all other pyrethroids were significantly less than 1000 pg/l.

In addition, we determined pyrethroids in 165 samples from 94 sampling sites of a project which intended to give a broad overview on the pollution status of smaller Bavarian rivers. Esfenvalerate was never detected, while β -cyhalothrin, cypermethrin and deltamethrin were detected just once. In consequence, only bifenthrin and permethrin were determined regularly above LOD at 9 respectively 33 of the 94 sampling sites with total detection frequencies of 7.8% and 34% and maximum concentrations of 1300 and 1700 pg/l.

In total, our monitoring data represents one of the first data sets that enable a preliminary assessment of pyrethroids in Bavarian surface waters. While none of the pyrethroids exceeded the proposed acute EQS values, further analytical development is required to lower the LODs for the assessment of chronic EQS values for all pyrethroids.

3.03.P-Mo198 Occurrence, Spatial Distributions, and Influencing Factors of Neonicotinoid insecticides in Soils from Agricultural Farmlands in China: A National Study

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Neonicotinoid insecticides (NEOs) are widely used in crop production in China, raising concerns about their health and ecological risks due to persistent residues in agricultural soils. Previous research has reported NEO contamination in soils of China's coastal regions, but national-scale data and information on NEO metabolites remain limited. On the other hand, both NEOs and microplastics (MPs) are commonly found in agricultural fields, but their co-occurrence characteristics under realistic fields have not been reported.

This study found that at least one NEO was detected in all soil sample. Imidacloprid was the most common parent NEO, with imidacloprid-urea as its primary metabolite. Results showed higher NEO concentrations in coastal areas at similar latitudes compared to inland regions. The extent of agricultural film cover (i.e., greenhouse, farmland with film mulching, and farmland without film mulching) and microplastics (MPs) might influence pesticide residues in soil. This study revealed an association between MPs and NEOs in realistic agricultural settings. Random forest models offered more reliable predictions than linear models and indicated that MPs, farmland type, and total nitrogen are key contributors to NEO levels.

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3.03.P-Mo199 Analysis of the Contamination and Reduction of Pesticide Residues During Transition from Conventional to Organic Rice Production in the Mekong Delta of Vietnam

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Rice cultivation in the Mekong Delta of Vietnam plays a critical role in both food security and the national economy. However, intensive agricultural practices, including the widespread use of pesticides and

fertilizers, have raised concerns regarding environmental sustainability and human health. This study aims to evaluate pesticide residues in the Mekong Delta, with a focus on the three provinces Vinh Long, An Giang and Dong Thap, and assess the feasibility of transitioning from conventional to organic rice farming practices. Household surveys were conducted in the three provinces to understand farmers' pesticide usage patterns. Therefore, the farmers were asked for information about which pest dominates on their farm, which pesticides are being used, on the frequency of the pesticide usage, and their application dose. Additional information about major crop, crop rotation, number of harvest per year, and farm size were also provided. The surveys revealed a diverse array of 85 different pesticide brands corresponding to 51 active ingredients, with insecticides comprising the majority, followed by fungicides and herbicides. Additionally, soil and water samples were collected for pesticide residue analysis in rice fields in each province respectively. Pesticide residues were extracted using solid-phase extraction for water samples and accelerated solvent extraction for soil samples. For the analysis of pesticides a non-target screening was carried out using LC-MS/MS and the Quanpedia data base, which contains the information of 750 widely used pesticides. The non-target screening identified 74 active compounds belonging to the categories Fungicides, Herbicides, Acaricides, Insecticides. Moving forward, ongoing farmers survey s will help to assess regional variations in pesticide usage, while targeted quantification of select compounds will provide insights into contamination levels. Ultimately, this study contributes to our understanding of pesticide pollution in rice farming regions and informs strategies for promoting sustainable agriculture and safeguarding environmental and human health in the Mekong Delta.

3.03.P-Mo200 Pesticides Contamination in the Irrigation System of Arequipa, Peru

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Agriculture is the second-largest economic activity in Peru, playing a significant role in the country's exports. Yet, agricultural practices in the country rely heavily on using pesticides and other chemicals to enhance and secure food production, raising public health and environmental concerns. Therefore, in this study, we aimed to detect the presence of various pesticides in the irrigation system of Arequipa, Peru. A sampling campaign was conducted in May 2022, analyzing water samples from key areas, including the Chili River, water ponds, and the Aguada Blanca and El Pañe reservoirs. Pesticide analysis involved water filtration, solid-phase extraction (C18 SPE cartridges), and a quantitative LC-MS/MS workflow. A total of 15 pesticides were analyzed in 26 samples. Results revealed the presence of 7 pesticides not approved under European regulations, although none of them are restricted by Peruvian regulation. Furthermore, Peruvian water quality standards do not include the monitoring of these pesticides, highlighting regulatory gaps. The detected pesticides included diuron, tebuconazole, propiconazole, carbendazim, azoxystrobin, and imidacloprid, confirming contamination of irrigation water bodies. This contamination suggests the existence of risks to farming workers, to consumers and to the ecosystems directly associated with the irrigation system. Further ongoing work is applying suspect screening analysis to a larger number of pesticides and other organic contaminants. The findings denote the need for stronger pesticide monitoring and regulatory measures in Peru and serve as critical evidence for governmental institutions to improve risk management and environmental protection policies.

3.03.P-Mo201 Meter-Scale Heterogeneity of Soil Properties Shapes Residual Pesticides and Potential Risk in an Agricultural Field

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Conventional agricultural management often includes field-wide, mostly homogenous application of pesticides without considering the spatial heterogeneity of soil properties. The fate and residual concentrations of pesticides, however, vastly depend on the soil properties influencing their potential risk to the environment. While the fate of pesticide residues in given soils is generally known, there is still a lack of in-situ observation on the impact of meter-scale heterogeneity effects on residual pesticide concentrations and their associated potential risk. In an agricultural field (0.37 ha) we determined the meter-scale spatial heterogeneity of soil properties and correlated the data to pesticide residues and their associated risks by chemical analysis and high-throughput cell-based in-vitro bioassays.

The soil pesticides and their transformation products were identified and quantified by LC-MS/MS upon accelerated solvent extraction. The bioassays were based on the mammalian cell-based AhR-CALUX and the freshwater algal growth inhibition assay using synchronised cultures of the green alga *Scenedesmus vacuolatus*. Chemical analysis and bioanalysis are combined by the bioanalytical equivalent concentration and iceberg modelling approaches.

The study field consisted of highly variable and spatially distinct soil properties. Textures ranged from loamy sand to silty clay. Chemical analysis identified 50 pesticides with total pesticide concentrations of 45 to 581 nmol kg⁻¹ with loads in topsoil being twofold higher than in subsoil. Pesticide concentrations varied significantly between soil types, with higher levels in silty loam than in loamy sand. As for pesticide residues, the bioassays revealed a high and spatially heterogeneous distribution of toxicity effects.

While predicted mixture effects by the detected pesticides in the microalgae test adequately explained the measured mixture effects, measured pesticide concentrations were only partially able to explain the high toxicity found by the AhR-CALUX bioassay. This indicates that other compounds, such as PAHs, that were also detected in the soil extracts, may be responsible for the observed activation of the AhR receptor. Our study shows that meter-scale soil properties influence the load and fate of pesticides and other compounds. This suggests that meter-scale heterogeneity should be considered in agricultural field management to minimize risks arising from potential pesticide accumulation or runoff.

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3.03.P-Mo202 GB Stereoisomer Guidance: Experience of Applying the Guidance to an Active Substance Assessment

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The EU has adopted new guidance on risk assessments for active substances (a.s.) and metabolites of plant protection products that contain stereoisomers (EFSA, 2019). HSE has considered the guidance and concluded that it should be adopted for use in GB. However, HSE has also developed additional GB guidance to supplement the EFSA stereoisomer guidance. The EFSA guidance is clear in that, as a general principle, stereoisomers need to be treated as separate substances with respect to the risk assessment. However, the need to treat stereoisomers as separate substances may lead to more complex risk assessments, where each stereoisomer is taken through its own individual exposure assessment, before information on total exposure levels is considered in the risk assessment. Given the potential complexities associated with stereoisomer-specific risk assessments, HSE considers that the more complex assessment approaches should be triggered only when necessary.

This poster demonstrates experience gained from applying this GB guidance for the first time to an a.s. assessment. This a.s. assessment presents additional complexities of a chiral parent that shows isomer specific degradation, that also forms a set of chiral metabolites that similarly show isomer specific degradation. Difficulties arose when applying the stereoisomeric excess concept to metabolites using parent dosed degradation studies. Where there are shifts in metabolite ratios from parent dosed studies, and the parent also degrades preferentially, it is hard to separate out how much of the change is due to the metabolite isomers and how much is due to the parent behaviour. This aspect is not currently addressed by available guidance, but in this case, HSE put more weight on the behaviour in metabolites dosed studies. The GB guidance introduces some pragmatism and helps simplify the approach to risk assessments considering enantiomers. Individual soils might demonstrate a change in isomeric excess and therefore technically should result in a stereoisomer-specific risk assessment. However, by considering the combined data from several soils it may not be necessary to conduct a separate risk assessment for each isomer. If there are small differences in the combined data, then evaluators could consider conducting a single exposure assessment for the sum of isomers. Additional supporting evidence from isomer specific leaching assessments were used in the GB case to simplify future product assessments.

3.03.P-Mo203 Worked Examples for the Proposed Revision of the Aged-Sorption Guidance Document

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In the EU Aged Sorption Guidance the use of field DegT50 values in combination with aged sorption parameters is only possible to a very limited extent. If the leaching assessment for a plant protection product (PPP) is based on field DegT50 values only, it is not recommended by the current guidance to

derive a DegT50equilibrium (rescaling of DegT50) for input in exposure models in combination with aged sorption parameters (fNE, kdes) as described for laboratory DegT50 values. This virtually prevents the mitigating effect of aged sorption on leaching and limits the applicability of the aged sorption guidance in combination with field DegT50 to very few cases.

Additional field studies were evaluated to show the relevance of aged sorption in the field: For evaluation of the field dissipation data, site-specific soil characterisation and daily climatic data for each trial were used to parameterise the FOCUS PEARL leaching model to allow detailed simulations of the transport of the PPP considered. These simulations using the site-specific data were used in multiple ways inverse-optimisation of DegT50equilibrium (ECPA06, 50 and 51), simulation of total residues (ECPA06, 50 and 51) and depth profile [P5-metric] analysis (ECPA06, 50 and 51). These detailed evaluations of the ECPA06, ECPA50 and ECPA51 datasets have illustrated the relevance of aged-sorption under field conditions.

Revisions to the current EU Aged Sorption Guidance fully allowing for the combination of field degradation and aged-sorption data proposed: If field and laboratory datasets are from different populations according to the EFSA DegT50 endpoint selector, then checks are made [P5-metric, Kd_app] to test if there is evidence of aged-sorption under field conditions. If there is evidence, then the geometric mean normalised field DegT50equilibrium is used in combination with the mean laboratory aged sorption parameters, fne and kdes, for the field soils. If there is no evidence of aged sorption under field conditions then field data and laboratory aged sorption parameters should not be used in exposure assessments.

To illustrate the proposed changes to the aged-sorption guidance document, worked examples for calculation of the P5 metric and inverse-optimised field DegT50equilibrium are presented.

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3.03.P-Mo204 xCropProtection: A Spatiotemporal Component to Simulate Prospective and Retrospective PPP Applications in Real World Landscapes

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There is currently both scientific and public discussion about chemical crop protection uses and trends. There seems to be consensus that chemical crop protection should be generally reduced or used more targeted in the future. In parallel there are requests for more realistic and holistic risk assessments of pesticides. We developed a spatiotemporally explicit component for simulating plant protection product (PPP) applications in landscapes which integrates with a larger spatiotemporal landscape model (xLandscape). This component, xCropProtection, can simulate applications at a field scale given PPP application information including target crops, spatial landscape information, and application periods. The result of running this component is a multidimensional dataset of PPP applications specific to individual fields and time steps. xCropProtection is parameterized with XML files, a file type used to store data in a hierarchical format well suited for sending and storing data. These XML files contain target crop types, PPP (or active substance) names, tank mixes, application rates (as a probability distribution function or a static value), probabilistic application windows, and mitigation measures for reducing exposure. xCropProtection functions both prospectively and retrospectively where input files can come from GAP tables in established risk assessment, can represent recommendations from plant protection advisory services, records from monitoring and field studies, or can be derived from market share grower surveys. xCropProtection is one component in the larger xLandscape framework that provides the simulation of more realistic pesticide applications for exposure, effect and risk assessments.

3.03.P-Mo205 Is a Common Environmental Risk Assessment Approach for Non-Professional Uses Possible Across Europe?

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The authorisation process of plant protection products (PPP) in the EU is regulated through Regulation (EC) 1107/2009 and its implementing daughter Regulation (EU) 546/2011 (uniform principles), both designed to lay down rules for PPPs for their authorisation and placing on the market in the EU.

Approaches for environmental risk assessment (ERA) developed in the frame of these Regulations predominantly address professional uses of PPPs, i.e., uniform full-area treatments of agricultural fields. Hence, the criteria for the authorisation of PPPs for non-professional uses (NPU, small scale often partial area treatments by amateurs) in the EU are based on these approaches, sometimes combined with specific exposure scenarios. In general, the authorisation of PPPs for such uses is fully relegated to information

available at the Zonal and/or National level. This results in Member States (MS) developing their own exposure scenarios and finally, different widely not harmonised approaches, as MS are ultimately responsible for approval and use of PPPs for NPU.

In this way, the absence of harmonised guidance for NPU can result in the authorisation of a certain PPP in one MS and the rejection in another, even if similar approaches were used. Additionally, the evaluation of the PPP dossiers for NPU tends to lengthen as the ERA is based on (inappropriate) evaluation criteria originally created for professional uses. This can hamper and delay the registration of PPPs for NPU, especially in case risk mitigation measures are indicated, ultimately leading to a lack of PPPs for non-professionals on the market.

This work aims to compile the current state of the art of ERA developed by EU MSs and Zonal Steering Committees for the evaluation of PPPs for NPU and to identify approaches and/or components which can contribute to a harmonised ERA for NPU in the EU. Most promising approaches currently used at MS level are (1) simplified authorisation of NPU products previously authorised for professional use, (2) restriction to 500 m² of applied area considering correction factors if home gardens/allotments are concerned, (3) small-sized packages, and (4) tailored risk assessment in case of ready-to-use (RTU) products, spot application, or indoor/greenhouse applications. All these efforts should contribute to a sustainable use of PPPs by providing appropriate risk assessment approaches and facilitating authorisation of most suitable PPPs.

3.03.P-Mo206 A Hidden Potential of National Registers of PPP Authorizations – Generalized Application Patterns

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EU has sophisticated legislation for PPP registration, but there is a lack of tools for the post-registration phase of pesticides, which is crucial to setting sustainable PPP use strategies and measuring their efficiency. Considering the goals of EU Farm to Fork, there is an urgent need for novel approaches to modelling pesticide exposures and risks at national and European scales.

Within the EU H2020 project SPRINT, we developed tools utilizing the data from national registers of PPP authorizations to model Generalized Application Patterns (GenAP) of pesticides. GenAP shows the authorized applications of each active substance on a defined crop in the defined time window, together with the application rate, BBCH range, and the maximum number of applications. The first GenAP was programmed in SQL for the relational database containing the Czech national PPP register. Many challenges were sorted out, including BBCH extraction from text notes, unit recalculation, and automated translations to English.

After an inventory across EU countries, we found that many countries have PPP registers with poor data quality, quantity, standardization, interoperability, and accessibility, hampering their use for GenAP. Thus, cooperation between the SPRINT project and the LexAgri database HOMOLOGA started. HOMOLOGA contains standardized data on registered PPP for all European countries. We developed SQL code for GenAP based on HOMOLOGA data. The benefit of this novel GenAP(H) is that it can be applied for any requested country because HOMOLOGA data are always structured the same way and use standardized ontology.

Within the SPRINT project research, GenAPs are further employed in the tools predicting the occurrence and risk of pesticides in soil, water, and air at local, regional, country and European levels. Thus, GenAP is combined with national PPP use statistics, national crop maps, and environmental variables. The benefit is that this approach is independent of monitoring results or farmers surveys. The resulting maps can identify the spatial-temporal cumulation of hazardous exposures and unacceptable risks, which is helpful in prioritizing pesticides for withdrawal and substitution.

It can be recommended that EU legally requests harmonizing the content and format of national PPP registers. If this happens, the huge potential of existing PPP-related data will be utilized for tools supporting EU efforts to reduce PPP use and risks.

3.03.P-Mo207 A Novel Approach for Large GAP Screening in View of EU Member State Authorisation – Extension for Groundwater and Drinking Water Treatment

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While the regulatory processes are largely harmonized over the three European zones, differences remain between the regulatory requirements of the EU member states (acceptance of different FOCUS-scenarios, mitigation options, required surrogate crops, application frequencies, accepted models). This makes the

evaluation of large GAP- (Good Agricultural Practice) tables (active ingredient submitted in multiple products, crops, and member states) very complex. The investigation which potential uses may pass the risk assessment and if a required mitigation is acceptable from the applicant's management and marketing perspective may become time consuming and expensive.

With the aid of the modelling software efam (environmental fate automated modeling) developed by knoell it is possible to automate model parameterisation and simulations for large GAP-tables utilising the relevant FOCUS-models and transfer the outputs to formatted tables as needed. Hence, with efam performing large sets of FOCUS runs for scouting becomes efficient and easy.

Previously we introduced a new tool deployed as an additional post-processing routine that is capable of analysing huge amounts of single PEC-values automatically for FOCUS surface water calculations. The routine scans efam result tables and groups PEC-values according to pre-defined matrices of FOCUS scenarios and mitigation options per country, as well as required surrogate crops. It adequately considers multiple/single applications, surface water bodies, and crop seasons. Finally, reliable passing rates for each modeled use with the active ingredient and multiple products are calculated via linear extrapolation for each member state and mitigation option. This allows a quick and reliable overview of potential maximum application rates in each member state.

Now an extension to FOCUS groundwater calculations and evaluation according to the EFSA Drinking Water Treatment guidance is available. For groundwater the tool is able to automatically analyse metabolic pathways implemented in FOCUS PELMO, PEARL and MACRO. It also allows for merging PEC-values from metabolites which occur in multiple PELMO pathways. For Drinking Water Treatment the evaluation is based on the time weighted average value at day 4 and considers evaluation of active substance and all relevant metabolites.

3.03.P-Mo208 FOCUS MACRO 5 Multithreading for FOCUS SW and GW Modelling

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FOCUS MACRO is used for SW and GW modelling for pesticide registration in the European Union. One single MACRO scenario simulation usually runs for 5 to 20 minutes, depending on the underlying hardware. The current official FOCUS MACRO model software package is only able to run one MACRO simulation after another. If a metabolite is included, the simulation time increases by a factor of 3, taking into account the intermediate and metabolite runs.

In order to improve the user experience, a simple multithreading tool (MAMA) was developed, that allows to reduce the simulation time to a minimum. No adaptations to the MACRO model or to the input data were necessary.

By using a state-of-the-art computer, up to 12 to 24 parallel runs are usually possible. Therefore, several run combinations could be performed in parallel what would reduce the simulation time by 90%. The MACRO multithreading tool also directly transfers the MACRO surface water bin output files to m2t files, needed as input for FOCUS TOXSWA. Moreover, with the MACRO 5 multithreading tool the user is able to de-select redundant FOCUS MACRO groundwater runs, i.e., parent and intermediate runs, saving additional simulation time.

It is intended to provide the multithreading tool to the community for beta testing, likewise it was done for the MACRO multithreading functionality for the CRD Higher-tier drainflow tool.

3.03.P-Mo209 Establishing a Link Between Calendar Dates, Degree-day and BBCH Stages of Relevant Crops Across the EU

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To advance the environmental risk assessment of plant protection products effectively, it is crucial to assess the relationship between crop phenology (i.e., Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie, or BBCH crop growth stages), calendar dates and locations across the European Union. The project we present, and currently ongoing under a framework partnership agreement between Wageningen University and Research, and the European Food Safety Authority, focuses on linking crop development stages, expressed using the BBCH scale, with calendar dates and degree-days for selected crops (soft wheat, durum wheat, spring barley, grain maize, rye, sugar beet, potato, field beans, winter rapeseed, and sunflower) across European agricultural regions. This approach aims to improve exposure assessments by incorporating regional variability and temporal dynamics into environmental risk assessment models.

The open-source CropLife Europe Crop Development Database, C2D2, which compiles crop growth data

from efficacy trials across Europe (2000-2020), will be critically evaluated and expanded with additional phenological data. The resulting enriched database is transformed into a grid-based format that covers European Union agricultural regions. Key BBCH stages, such as emergence, flowering, and maturity, are calibrated and validated against observed data, offering detailed, spatially resolved insights into crop development patterns. This work advances the environmental risk assessment by supporting: improved estimation of pesticide application dates, better predictions of off-target exposure, including spray drift and pollinator risks, through accurate timing of crop development stages, enhanced scenario development for the European Union regulatory frameworks, such as FOCUS surface water models and bee exposure scenarios. Additionally, the results of this project could be used to update existing crop growth models, such as those used in the Joint Research Centre Monitoring Agricultural Resources project, to generate crop height as a critical parameter for spray drift assessment. By addressing the objectives, this project can provide a robust foundation for improving environmental risk assessment methodologies, supporting the regulatory processes, and promoting consistent and scientifically sound exposure assessment for pesticide use across the European Union.

3.03.P-Mo210 Improved Soil Fumigants Exposure-Related Data and Mitigation Measures Efficacy of Different Fumigation Strategy and Techniques

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Pressure exerted by soil-borne plant pathogens in some area for certain high value crops is a problem of growing concerns. Several approaches have been considered for their control but the dependence on chemicals products to assure food security (and also food safety) still exist and recently, especially for root-knot nematodes and cyst nematodes, this issue is no longer confined to southern areas but is also emerging in some areas of northern Europe.

Fumigation is an activity to control such pathogens, but fumigant products may pose humans and environmental safety concerns and must be properly managed to maintain a sustainable intensification of production. This aspect must also be reconciled with the ambitious goals of the EC objectives.

To balance the importance of the contribution of chemical pesticides to food security, and social need of chemical pesticides use reduction required, sustainable and integrated nematode management strategies, taking into account the mitigation measures considered to reduce exposure risk are fundamental. Within this framework, exposure assessment is a critical component and the lack of EU soil fumigants specific guidance on exposure and risk assessment, has resulted in regulatory uncertainty.

As an example, (1) pesticides applied as soil fumigants are very volatile and FOCUS GW models are not able to simulate adequately the volatilization from the soil surface and (2) the mitigation measures, that focused on developing fumigation methods using low-permeability tarps, including totally impermeable film (TIF) or (3) context specific application techniques/strategies, as soil moisture management, that might have a relevant impact on potential exposure need to be considered during the risk assessment and decision making.

In this work improved fumigants exposure-related data and mitigation measures efficacy of different fumigation strategy and techniques are provided to support both the identification of proper models input data both the ongoing national and international strategies for a proper adoption of soil fumigation procedures that might significantly increase fumigant efficacy having potential beneficial effects on fumigant air emissions and breakdown residues, and necessary to reach the level of exposure reduction set at the EU level.

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3.03.P-Mo211 Establishment of a Simultaneous Analytical Method for 370 Pesticides and Metabolites in Honey Using LC-MS/MS

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Honeybees (*Apis mellifera* L.) can be exposed to pesticides during plant pollination, increasing the likelihood of pesticide residues in honey, thus necessitating continuous monitoring. A simultaneous multiresidue analysis method is particularly needed for neonicotinoid pesticides and their metabolites, which are suggested as potential contributors to Colony Collapse Disorder (CCD). This phenomenon was first observed in the United States and later reported in many European and Asian countries. This study optimized sample preparation using 1 g of honey by comparing combinations of two extraction solvents (acetonitrile and 0.1% formic acid in acetonitrile) and two QuEChERS salts (Original vs. EN 15662). The

Multiple Reaction Monitoring (MRM) mode of a Shimadzu LCMS-8040 was utilized, and the separation of analytes was conducted using a Cadenza CD-C18 (3 μ m, 150 $\times$ 2 mm) column. It was found that superior recovery rates were provided by the combination of 0.1% formic acid in acetonitrile with the Original QuEChERS salt. In the method validation using the established analytical method, the limit of quantitation (LOQ) was determined to be 0.01 mg/kg for 329 of the 370 pesticides analyzed. Calibration curves showed correlation coefficients (r^2) 0.980 for 348 analytes (94.1%). At a fortification level of 0.01 mg/kg, 266 pesticides (71.9%) showed recovery rates between 60-120% (CV 32%); at 0.1 mg/kg, 349 (94.3%) showed recovery rates of 70-120% (CV 22%). Matrix effects indicated that 78.1% of the analytes were within the soft range ($\pm$ 20%). Meanwhile, 11.6% were in the medium range (25% to 20% or +20% to +50%), and 10.3% were in the strong range (below 25% or above +50%). The analysis of 14 neonicotinoids, comprising 7 pesticides (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, thiamethoxam), 3 dinotefuran metabolites (-UF, -MNG, -DN), and 4 imidacloprid metabolites (-urea, -olefin, 5-hydroxy imidacloprid, 6-chloronicotinic acid), demonstrated a LOQ range of 0.0025-0.1 mg/kg. The calibration curve correlation coefficients (r^2) were 0.980, with recovery ranges of 75.6-123.4% at 0.01 mg/kg and 26.4-120.2% at 0.1 mg/kg. The analytical method established in this study for honey is expected to facilitate the simultaneous monitoring of 370 pesticides and their metabolites.

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3.03.P-Mo212 Novel Triple Quad Approaches for Sensitive Quantification of > 1000 Pesticides in Single Runs

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Pesticides represent the most frequently analyzed hazardous compounds in food and feed globally. A key challenge is the analysis of a vast and ever-increasing number of diverse multi-class pesticides in single runs. The demand for higher sample throughput on top, screening techniques gain popularity, particularly for organic and pesticide-free products, to push rapid detection while still allowing for quantification of positive samples. Proposed here is a quantitative analysis method for a large number of pesticides in food using a novel triple quadrupole MS. Both throughput and the high number of target pesticides will be addressed. For the evaluation of the analytical performance, the new method was optimized in a real scenario in common matrices for more than 500 pesticides combining positive and negative ions. The aim is to evaluate in detail how the speed, number of compounds and other parameters influence the sensitivity and robustness in short- and long-term work.

An Elute 2 UHPLC was used with water (A) and methanol (B) (both (0.01% FA, 2 mM ammonium formate) at a 15-100% B gradient in 0.5-8 min. Runtime was 9.2 min with subsequent 2.5 min equilibration. 2 μ L sample were injected on a Bruker Intensity Solo 2.0 100 $\times$ 2.0 mm column at 45 $^{\circ}$ C. An EVOQ-DART TQ+ with pulser-HESI was used in MRM Compound Based Scanning. At a scan time for each compound of 5-11 ms, the method that can accommodate >1000 pesticides in fast polarity switching with 2 transitions in a single run. A probe temperature program reduced the impact for thermolabile compounds eluting at the chromatogram end (Abamectin, Ivermectin, Sprimesifen). A calibration curve containing 520 mixed pesticides was prepared in pepper matrix (QuEChERS) with five calibration levels at 0.2-50 ppb. 30 real samples prepared with the same extraction method were injected afterward to quantify the potential pesticides.

The calibration curve showed $R^2 > 0.99$ for 95% of the compounds with ion ratios at < 10% deviation. For 10 consecutive injections, an RSD < 10% for most pesticides was achieved in the real matrix spiked with 2 ppb in total. Different methods have been concluded for 520 pesticides in a single run (positive/negative ionization). Due to novel electronics and the rapid polarity switching, the intensity and ion ratio remained consistent regardless of the scan speed. All these parameters indicate a robust and reliable method for screening and quantitation up to 1000 pesticides in a single run.

3.03.P-Mo213 Determination of Pyrethroids in Water Samples According to the EU Water Framework Directive Using Atmospheric Pressure Gas Chromatography Tandem Mass Spectrometry (APGC-MS/MS)

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Reliable and highly sensitive analytical methods are needed for detection, quantification, and identification of pyrethroids water samples. In the proposal of directive amending Directive 2000/60/EC establishing a framework for Community action in the field of water policy and Directive 2008/105/EC on environmental quality standards (EQS) in the field of water policy the EQS for cypermethrin and its isomers are more stringent and with bifenthrin, deltamethrin, and permethrin new pyrethroids were added. The assigned annual average EQS in surface water go as $1.7 \mu\text{g/l} \times 10^{-6}$ for deltamethrin which is an analytical challenge for water testing labs.

This work describes the sample preparation and analytical method using APGC-MS/MS to be able to reliably identify and quantify pyrethroids even at the very low EQS that can be expected from the new proposal. APGC ionization is used in the application because it is considered soft when compared with techniques such as Electron Ionization, which means that less fragmentation is observed for many compounds. Reduced fragmentation simplifies pre-cursor ion selection, subsequently resulting in higher sensitivity and specificity in MS/MS analyses.

3.03.P-Mo214 QuEChERS-Based Optimization and Application of Broflanilide Analysis in Persimmon Flesh, Peel, Peduncle, and Fully Ripened Fruits for Assessing Washing Efficiency

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Broflanilide is a meta-diamide pesticide that exerts a strong insecticidal effect by non-competitively acting on GABA (gamma-aminobutyric acid) receptors. These receptors function as inhibitory neurotransmitters in the central nervous system, thereby paralyzing pests. Broflanilide is widely used in persimmon farms to control the Persimmon Fruit Moth (*Stathmopoda masinissa* Meyrick). Many consumers wash agricultural products in various ways, such as rinsing, scrubbing, or soaking, to mitigate pesticide exposure. The effectiveness of these washing methods in reducing pesticide residues varies depending on the washing solution, technique, and the part of the produce being treated. This study optimized the analytical method for different parts of persimmons using the QuEChERS method and applied it to compare the residual levels of broflanilide across various washing methods applied to these parts. A method showing excellent sensitivity, accuracy, and precision, as well as low matrix effects was established by comparing purification conditions across persimmon flesh, peel, peduncle, and fully ripened fruits. The established method had a LOQ (limit of quantitation) of 0.01 mg/kg , with a correlation coefficient above 0.990 for calibration curves analyzed using matrix-matched standards. Recovery tests met acceptable criteria (70-120% recovery, relative standard deviation $\leq 20\%$), confirming its reliability. This analytical method was used to compare the residual levels of the pesticide across different washing methods and tissue types in persimmons immersed in a spray solution made with a 5% broflanilide agrochemical product (water-dispersible granule; WG). The washing methods included rinsing the persimmons under running tap water, gently rubbing under running tap water, and immersion in washing solutions containing 1.0% acetic acid, 0.2% polyethylene glycol 4000, or 10% sodium bicarbonate (NaHCO_3), followed by rinsing with running water. The results were analyzed separately for each part, and the washing efficiency (%) was calculated by comparing them with the untreated control. This research provides insights into strategies for mitigating pesticide exposure and foundational data for future studies on measuring pesticide exposure through residue patterns across different parts of persimmons.

3.03.P-Mo215 Determination of Pesticide Residues in Fiber and Cottonseed in Major Cotton (*Gossypium hirsutum* L.) Production Regions of Türkiye and Assessment of Risk to Consumers

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Cotton is an important cash crop, mainly used in the textile industry. Cotton is cultivated on about 32.88 million ha in more than 60 countries worldwide, with an annual production of about 73.74 million tons of seed cotton. Although cotton planting covers only 2.80% of the world's agricultural area, it meets about 27% of the global textile industry demand. Many diseases, pests, and weeds damage cotton plants and can cause significant yield losses. Pesticides are used widely to control pests, diseases, and weeds. Pesticide use poses risks to the environment, biodiversity, and animal and human health via the food chain. In this study, pesticide residues were investigated in Şanlıurfa, Diyarbakır, Aydın, İzmir, Hatay, and Adana provinces, where cotton production is intensive, and pesticide residue dynamics were determined in both fiber and seed. Samples collected from each province were quickly transferred to the laboratory and stored at -20°C until extraction and analysis. Pesticide analyses were conducted using the QuEChERS method, and pesticide residue analysis was performed using GC-MS (gas chromatography-mass spectrometry) and LC-MS/MS (liquid chromatography-mass spectrometry) instruments. Risk modeling with the 'PRIMo (Pesticide Residue Intake Model) revision 3' model of the European Food Safety Authority (EFSA) will be performed by considering the toxicity of the mixture of pesticide residues. In addition, risk classifications

will be calculated based on data from Türkiye and WHO. The results of this study will be used to plan better pesticide management for more sustainable cotton production and integrated pest management. The results of the project will contribute to environmentally friendly spraying programs that support food safety.

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3.03.P-Mo216 Fate and Transport of Microplastics and Micropollutant in Soil Environment

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The unique strength and resistance to water, shock, and electricity makes plastic a versatile material and has many valuable uses. As more the addiction towards minimal/single-use or disposable plastics, the environment ends up with severe consequences. Microplastics (MP), plastic size <5mm, elevates alarms about the ecological and health risks worldwide. Miserably, the massive practice of plastic goods and mismanagement of plastic waste disposal leads to the MP pollution in aquatic water bodies and their ecosystems. Similarly, the aquatic environment is contaminated with micropollutants that are sourced from industrial effluent, agricultural discharges and partially treated domestic wastewater. Even if MP doesn't cause acute fatal effects on living organisms, they can act as the carrier of these micropollutants, and cause root chronic toxicity, which is considered as a key issue in long-term exposure.

In this study, the soil sample was collected from Kandi Lake ecosystem, Kandi, Sangareddy Telangana, India and characterized for geotechnical and hydrodynamic properties. Continuous flow column experiments were conducted in one-dimensional column simulating the situation pertaining to the actual lake water infiltration and relative transport of microplastics and the selected micropollutant 2,4-dichlorophenoxy acetic acid (2,4-D) to represent their movement from the lake to sediment and soil ecosystem. The flow rate and the varieties of the MP were considered as the study factors and the results were interpreted for the fate and transport of MP from the lake to the soil ecosystem. This study enlightens the fundamental knowledge of the behavior of microplastics and micropollutant in the soil ecosystem and gives deeper insights into the vector behavior of the microplastics. This study opens a door to the possible transport and co-transport mechanism involved in the transport of microplastics and micropollutants in the natural ecosystem.

3.03.P-Mo217 Dissipation Pattern of Spirotetramat During Cultivation of Agricultural Products for Establishing Post-Harvest Residue Level in Korea

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This study examined the dissipation patterns of pesticide residues in agricultural products during cultivation and proposed pre-harvest residue limits (PHRLs) based on these patterns and biological half-lives. Pesticides were applied to agricultural products in three different regions at the recommended dose. Samples for the dissipation pattern study were collected at intervals of 0, 1, 3, 5, 7, 10, and 14 days post-treatment and analyzed using LC-MS/MS. After extraction and purification of insecticide residues via the QuEChERS method, the samples underwent quantitative analysis by LC-MS/MS. The recovery rates for the tested insecticides ranged from 71.7 to 113.1%, with limits of quantification at 0.01 mg/kg and coefficients of variation below 5%. Based on first-order kinetics, the dissipation half-lives of the combined spirotetramat and spirotetramat enol were calculated as 6.3, 4.8, and 6.3 days, respectively, across the different regions. The biological half-lives of pesticides were determined for each field study.

Consequently, a PHRL was recommended for 10 days before harvest to ensure safe residue levels in the agricultural products.

3.03.P-Mo218 Flood Mediated Pesticide Uptake into Riparian Plants and Phytophagous Aphids

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Surface waters are known to be polluted with various compounds including synthetic pesticides. Pesticides are able to move between aquatic and terrestrial ecosystems, e.g., via flood mediated contaminant transport from water to land. As a bordering habitat, the riparian zone functions as a diversity hotspot for many plant and insect species. Therefore, the exposure and uptake of flood-mediated pollutants by riparian

soil, riparian plants, and non-target arthropods requires evaluation. We therefore conducted a climate chamber pot experiment employing potted stinging nettle and the common nettle aphid. Each pot was artificially flooded four times within 24 days with flood water containing 31 insecticides and fungicides at an environmentally relevant concentration. We measured the concentrations of the pesticides in soil, plants, and aphids using HPLC-MS/MS. The concentrations in soil and nettle increased significantly with each flood, leading to a six-fold increase in sum pesticide concentration in soil, and a thirty-fold increase in sum pesticide concentration in nettle leaves and stems. Twelve pesticides were detected in the phytophagous aphids after four floodings, four of which were biomagnified compared to the concentrations in plant material. As aphids and nettle are a widespread food source within the riparian zone, the observed trophic transfer of contaminants might pose further risks to other arthropods such as pollinators, via honeydew, or to predators of aphids, such as spiders. Therefore, we propose to include the flood-mediated contaminant pathway as a potentially relevant exposure pathway within riparian areas, which is a habitat also experiencing exposure via various other exposure pathway routes. This is especially relevant in the light of the expected future increase in flooding intensity due to climate change.

3.03.P-Mo219 Blooming Concerns: Evaluating Exposure Toward Pollinators from Ornamental Plants

Mafalda Castro¹, Natalie Julia Ruddle², Pelagia Xirogiannopoulou³, Helen Thompson², Alexander Blakey², Jorge Eduardo Yanez Heras⁴, Beatriz Anton-Garrido⁵ and Helen Tungate², (1)Syngenta Nordics A/S, Strandlodsvej 44, 2300 Copenhagen, Denmark, Denmark, (2)Syngenta, Jealott's Hill International Research Centre, United Kingdom, (3)Syngenta, Jealott's Hill International Research Centre, Greece, (4)Eurofins Agroscience Services EcoChem GmbH, Germany, (5)Eurofins Trialcamp S.L.U., Spain Ornamental plants are an extremely diverse group of plants, varying from small herbaceous plants to large ornamental trees. In addition to their diversity, their commercial cultivation is often associated with non-conventional applications of plant protection products (PPPs) and cultivation methods, thus presenting unique challenges in environmental risk assessment. In such cases, traditional approaches to assessment of exposure may not accurately reflect the potential impact on non-target organisms, particularly pollinators and arthropods. Typical pollen/nectar residue studies involve the widely used PPPs application via foliar or seed treatment. Unlike typical residue studies, this study involves the less-studied residue profiles of different ornamental plants through a soil drench application and non-soil bound cultivation, often regarded as more environmentally friendly since environmental exposure is limited. This study aims to evaluate the accuracy of conventional environmental exposure assessments for ornamentals by: (1) growing various ornamental plants in protected conditions, (2) applying a slow degrading, systemic plant protection product via soil drench (1000 g/ha), and (3) sampling pollen, nectar, and leaves during flowering. Although in pollen/nectar residues studies spatial variation is often used to obtain a certain percentile (usually 90th), given that the application in this case consists of a non-soil bound plant protection product, inter-species variation is expected to be greater than variation between test sites. This will allow exposure to be quantified from a PPP through plant matrices to pollinators and non-target arthropods. This approach aims to provide insights into how residues of PPPs are expressed in different ornamental species potentially improving risk assessment accuracy for these diverse plant types and cultivation methods. Preliminary results show very low residues of the plant protection product in the different plant matrices, even when compared to applications of the same PPP in field crops via seed treatment application.

3.03.P-Mo220 Development of Disposable Biosensors of Acetylcholinesterases from Leaf-Cutting Ants for the Detection of Potential Pesticides

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The application of chemical compounds such as organophosphates and carbamates as pesticides to control ants can have a negative impact on the environment and human health. In the present study, two electrochemical biosensors based on acetylcholinesterases from leaf-cutting ants were developed for the detection of insecticides in the field and potentially in plant extracts. The pests responsible for most damage and loss in agriculture are leaf-cutting ants (*Atta* and *Acromyrmex*). This insect causes damage in agricultural and forestry areas, mainly in monocultures, such as *Pinus*, *Eucalyptus*, *Gmelina* and *Citrus*. For the development of biosensors, the microelectrodes were screen-printed on polyester foil using carbon and Ag/AgCl-based inks. The acetylcholinesterase inhibition studies with organophosphate malathion reached detection limits of 9.1 nmol L⁻¹ for AChE A and 4.7 nmol L⁻¹ for AChE B. The extracts of *Solanum lycocarpum* and *Ocotea* sp. were applied for inhibition studies and provided different inhibitory activity profiles between themselves and for each enzyme. The extract of *Solanum lycocarpum* obtained

80% inhibition against AChE A, being a promising extract for future studies in the search for natural insecticides for leaf-cutting ants. In conclusion, the developed biosensors are new tools for screening extracts with potent insecticidal activities and detecting insecticide in the environment with effect to human health.

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3.03.P-Mo221 Occurrence and Spatial Distribution of Neonicotinoids in South Korean Honey and Pollen Samples

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Neonicotinoids, a class of systemic insecticides can accumulate in plant tissues including nectar and pollen, and subsequently enter the food chain through pollinators such as honey bees. These residues pose potential risks not only to pollinators but also to human health. However, data on neonicotinoid contamination in honey and pollen particularly in South Korea is limited. This study investigated the occurrence and spatial distribution of neonicotinoid residues in both honey and pollen samples across various regions of South Korea. A total of 79 honey samples and 27 pollen samples were collected from agricultural, mountain, and urban areas and analyzed for 18 compounds including neonicotinoids and their metabolites using liquid chromatography coupled with mass spectrometry. All honey and pollen samples contained at least one target compounds indicating widespread contamination. Among honey samples, acetamiprid (83.5%), dinotefuran (77.2%), and flonicamid (58.2 %) were the most frequently detected compounds while pollen samples showed high detection frequencies of acetamiprid (100%) dinotefuran (96.3%) and 5-hydroxy-imidacloprid (85.2%). Honey samples collected from agricultural area demonstrated higher concentrations of flonicamid (2500 ng/g), acetamiprid (258 ng/g), and imidacloprid (133 ng/g), compared to mountain and urban samples. Pollen sample analysis revealed different distribution patterns, with mountain regions showing high concentration of acetamiprid (349 ng/g) followed by flonicamid (154 ng/g). Furthermore, the dietary risk assessment for humans suggests that consuming a certain amount of honey is unlikely to pose a health risk due to neonicotinoid intake. However, the Risk Quotient values for imidacloprid to bees was determined to be 4.0, significantly exceeding the safety threshold of 1, highlighting the elevated risk of acute toxicity to honey bees. These findings demonstrate the widespread presence of neonicotinoid residues in honey and pollen, with complex spatial patterns influenced by local agricultural practices and environmental factors. The study emphasizes the need for continued monitoring and regulation to protect pollinator and human health from potential risks associated with neonicotinoid exposure.

3.03.P-Mo222 Building Age and Seasonality Impact Levels of Pesticides in Household Dust

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Pesticides are typically considered of concern in outdoor environments, but there is growing awareness of their potential contribution to exposure in indoor environments. While most pesticides are used in agriculture, domestic use is also widespread. We evaluated the exposure of the Czech children to both legacy organochlorine pesticides (OCPs) and currently used pesticides (CUPs) in their home environment, by sampling settled indoor dust, covering a diverse range of dwellings in urban, suburban, rural-agricultural and forested areas. Given the known seasonality in pesticide use and outdoor air concentrations of pesticides, we additionally sampled a subset of homes monthly over one year. Out of the 36 CUPs analyzed, 15 were detected in at least one individual dust sample, with atrazine (detection frequency = 24.1%), carbendazim (DF = 84.5%), propiconazole (DF = 49.1%) and tebuconazole (DF = 11.2%) detected more frequently. OCPs were detected in all homes. Pesticide concentrations were investigated in the context of geospatial and household variables: propiconazole, used as a biocide on building products, was found to be significantly higher in houses with adjacent gardens; while OCPs, especially DDT-related compounds, were substantially higher in homes more than 40 years old, indicating the continued presence of residues from past indoor use. Strong seasonality was also observed in the CUPs in dust, with levels in indoor dust 5-10x higher in spring in rural homes, aligned with the timing of outdoor pesticide application. The exposure estimates for various pesticides in children, under both median and high exposure scenarios, remain well below established safety thresholds, indicating minimal concern for potential adverse health effects in children for this exposure pathway.

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3.03.P-Mo223 Dissipation Patterns and Pre-Harvest Residue Limits of Pesticides in Ponytail Radishes in Korea

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The Republic of Korea's Ministry of Food and Drug Safety (MFDS) sets Pre-Harvest Residue Limits (PHRLs) to proactively address potential exceedances of Maximum Residue Limits (MRLs) in crops during pre-harvest steps. PHRL refers to the maximum allowable concentration of pesticides in the pre-harvest steps, by date (up to 10 days before shipment). Exceeding the PHRL during pre-harvest steps results in actions such as disposal, shipment delay, and purpose conversion. This study evaluated the dissipation patterns of pesticide residues in ponytail radishes during cultivation in greenhouse and established PHRLs based on the pesticides dissipation characteristics and biological half-lives. Pesticides and crops with a high frequency of unsuitability in the past were selected. Ponytail radishes (*Raphanus sativus* L.) are among the most commonly consumed vegetables in kimchi, following Korean cabbage and radish. Pesticides were applied at recommended doses following Good Agricultural Practices (GAP) across three different regions. Samples for the dissipation study were collected at 0, 1, 2, 3, 5, 7, and 10 days after treatment (DAT) to monitor residue levels over time. Residues were extracted using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method and analyzed by high-performance liquid chromatography or gas chromatography-mass spectrometry. The analytical method satisfied validation criteria for pesticide residues, achieving 70-110% recovery, relative standard deviations below 20%, and a limit of quantitation below 0.01 mg/kg. Following foliar application, pesticide residues in the leaves and roots of ponytail radishes decreased steadily. Biological half-lives for each pesticide were calculated using pseudo-first-order kinetics based on dissipation regression data from the three field studies. In accordance with guidelines from the MFDS in Korea, PHRLs were determined using the 95% upper confidence limit (95% UCL) of the dissipation constant (?) and MRLs for leaves and roots. Daily PHRLs for the 10 days before harvest were recommended to prevent agricultural products from exceeding MRLs at the point of harvest.

3.03.P-Mo224 Effectiveness of Home-Based Methods for Reducing Pesticide Residues on Grapes

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Despite EU regulations about maximum residual levels (MRL) on vegetables and fruits, pesticide residues can still be found on many produce items, in some cases exceeding the MRL. This has led consumers to use various home-based methods to remove pesticides and reduce their exposure. This study tested the effectiveness of such methods in removing pesticide residues of tebuconazole from grapes.

Grapes were treated with a common pesticide for fruit plant treatment, tebuconazole, with the levels 0.4 mg/kg (MRL for tebuconazole is 5 mg/kg). Grapes were then subjected to eight different home-based treatments, which covered washing by cold water for 5 sec and 30 sec and hot water and soaking in water and solutions of baking soda (sodium bicarbonate), salt (sodium chloride), vinegar (acetic acid), and potassium permanganate for 10 minutes. Pesticide residues were quantified on the grapes by QuEChERS method and HPLC-MS analysis.

Every home-based method reduced the tebuconazole levels with the range 30-70 %. Our results showed that simple water-based treatments were the least effective in removing tebuconazole residue. The lowest effectivity was observed with the 5 sec wash, however, the prolongation of the rinse time to 30 sec increased the effectivity to 46 %. The highest effectiveness of 71 % was observed for two options: soaking in solution of vinegar (2 % acetic acid solution), and baking soda (1 % solution of sodium bicarbonate). While home-based methods can reduce tebuconazole residues on grapes, their effectiveness varies significantly, and they do not eliminate them completely. It is important to note that these methods may not remove all pesticide residues similarly, because of their differences in chemical structures.

3.03.P-Mo225 Geospatial Analysis as a Toolbox for Identification of Antibiotics Sources in European Surface Water Bodies at a Catchment Scale

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The presence of antimicrobial drugs in the environment poses potential risk to ecosystem health due to toxicity to aquatic organisms, the potential to disrupt microbial communities, and contribute to the development of antimicrobial resistance (AMR) even at low or sub-lethal concentrations, representing a risk to human health. Antibiotics are released to the environment through wastewater treatment plants

(WWTP) and surface runoff. The European Union has added several antibiotics on the Water Framework Directive (WFD) watch list which identifies emerging pollutants that require monitoring to assess their environmental impact. This study focused on a catchment area within the UK, examining the water quality of surface waters that receive WWTP discharges. Using advanced geospatial modelling techniques, the study aimed to enhance our understanding of fluoroquinolone sources in the environment by analysing monitoring data specific to this catchment.

Discharge points of WWTP, proximities of hospitals and effluents from antibiotics manufacturing sites may correlate with elevated concentrations of antibiotics in surface water. To test this hypothesis, we combined Geographically Weighted Regression (GWR), Hotspot Analysis with Getis-Ord G_i^* technique and Kriging to identify and predict potential sources and distribution of the fluoroquinolone antibiotic ofloxacin concentrations in surface water. The spatial analysis approach offers a cost-effective method for predicting spatial and temporal dynamics of antibiotics pollution. By leveraging existing monitoring data, it bypasses the need for wide-scale sampling campaigns while assisting monitoring efforts. We anticipate establishing temporal and spatial relationships between the antibiotic's concentrations and key sources, as well as generating predictive maps of the levels and distribution of pollution.

This research will contribute to improved monitoring strategies and source identification methods for antibiotic pollution. By pinpointing major contributors to the environmental occurrence of ofloxacin, and predicting concentrations in unsampled areas, our findings will inform development of targeted sampling efforts and mitigation strategies, supporting WFD objectives of combating the spread of antimicrobial resistance in aquatic ecosystems in the UK and the EU and Member States.

3.04.A Scientific Advancements in the Fate and Toxicity of Metals: Data, Models, Tools, and Their Application in Environmental Regulations

3.04.A.T-01 Measuring Bioavailable Aluminium in Natural and Laboratory Waters

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Aluminium (Al) has been regarded as a difficult to test substance due to its formation of oxy-hydroxide compounds at pH values common in natural waters. The formation of gill reactive compounds occurs when acidic water ($\text{pH} < 5$) mixes with circumneutral pH water in natural ecosystems, or when alum is used in water treatment or in laboratory tests when a soluble Al salt is added to water. The formation of the hydroxides results in gill smothering, reduced oxygen transport and alteration of ion transport across the gill. In the laboratory, organisms often die due to oxygen deprivation. In nature, salmon smolts die when migrating to the ocean and cannot regulate the increase in salinity after exposure to Al or when sensitive organisms are exposed to mine effluents in mixing zones. Our 10-year aquatic research program has resulted in the development of a biotic ligand model (BLM) and a multiple linear regression model (MLR) to predict toxicity. The MLR model has been used by the USEPA to develop a water quality standard. Unlike other cationic metals, Al toxicity correlates with total metal in solution and not dissolved Al. Water quality guidelines in several countries require analytical measurements of total Al using a strong acid digestion. Natural water samples contain suspended solids (clays, minerals) with substantial amounts of natural Al. Thus, samples may not meet regulatory standards due to the presence of suspended solids. To alleviate this problem, we developed an analytical method that measures bioavailable Al without measuring the Al contained in suspended solids. The method was approved by ASTM in November 2024. We applied this method to samples from 10 rivers in the US. Our presentation will include details on the toxicity of Al, the MLR model and the analytical method for bioavailable Al.

3.04.A.T-02 Development and Application of a Cobalt Chronic Biotic Ligand Model

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This presentation will provide an overview of the influence of water quality parameters on the chronic toxicity of cobalt to aquatic organisms. The influence of various toxicity modifying factors (TMFs) were evaluated in an empirical testing program that used invertebrates (*Brachionus calyciflorus*, *Ceriodaphnia dubia*, *Daphnia magna*), fish (*Pimephales promelas*), and algae (*Pseudokirchneriella subcapitata*). Study results were used to develop a cobalt biotic ligand model (BLM). Important TMFs for explaining cobalt bioavailability and toxicity included pH, DOC, Ca, and Mg. The cations, Ca and Mg, have similar modes of action and can be addressed jointly as water hardness. Bioavailability effects were similar between taxa so a pooled model including fish, invertebrates, and plants/algae was judged to be best for guideline development. This also simplifies the normalization of a species sensitivity distribution (SSD) and could result in a critical accumulation value associated with the 5th percentile or guideline value, rather than the

need for normalizing the entire SSD for each new set of conditions. The resulting model was validated by comparing its performance against measured toxicity in several natural waters, selected to represent a range of physiochemical parameters typical of European and North American surface waters. Model performance in the natural waters validation dataset was comparable to that of the calibration data. The HC5, developed from an SSD of previously published chronic toxicity data, and the cobalt BLM was used to evaluate the affect of TMFs (i.e., pH, DOC and hardness) on potential regulatory values. The results show that chemical factors have an important role in determining cobalt toxicity, and that bioavailability models such as the BLM should be used to consider these factors in order to produce accurate water quality guidelines protective of aquatic life.

3.04.A.T-03 Influence of Water Chemistry on Silver Toxicity to Freshwater Algae: Development of a Chronic Biotic Ligand Model (BLM)

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Due to silver's unique properties, such as high heat and electricity conductivity, light reflectance and antimicrobial activity, it has been made indispensable in a variety of industries. With such widespread use, emissions to the environment are inevitable. This fact has positioned silver as a candidate priority contaminant under the Water Framework Directive, highlighting the need for scientifically derived environmental quality standards (EQS). EQSs for metals are often derived using bioavailability models, which are crucial because toxicity in water is influenced not only by the soluble metal concentration but also by water chemistry, which drives metal speciation and interaction with biota. While an acute biotic ligand model (BLM) already exists for silver in freshwaters, a chronic model has yet to be developed. This gap limits our ability to assess long-term risks posed by silver in freshwater environments. To address this need, we investigated chronic (72h) silver toxicity to freshwater alga *Raphidocelis subcapitata* in synthetic freshwaters, focusing on developing parameters essential for a chronic BLM. We univariately tested six water chemistry parameters, in their typical naturally occurring ranges, to determine their influence on silver toxicity. Results indicate that pH (range 6-8.7) and natural organic matter (range 0.5-22 mg DOC/L, DOC dissolved organic carbon) have a significant influence on EC10 values (silver concentration causing 10% reduction in algae growth rate), yielding differences in EC10s by factors of 4.5 and 27, respectively, across the selected ranges. A logarithmic relationship was observed between H⁺ and Ag⁺ free ion activities, suggesting the bioavailability of hydroxide complexes. Moreover, results indicate that chloride complexes are bioavailable within the range of 4-80 mg Cl/L, as toxicity was correlated with total silver concentration, rather than calculated free ion activity. This suggests that silver does not fully conform to the classical free ion activity model. Water hardness and sodium exhibited minimal or no influence on toxicity. These results offer essential parameters for establishing a chronic silver BLM for algae, making this work particularly relevant for regulatory purposes.

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3.04.A.T-04 Predicting Metal Toxicity in Aquatic Environments using an Innovative Toxicodynamic Model

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Within the framework of the French national biomonitoring program under the Water Framework Directive (WFD), water agencies measure bioaccumulated concentrations of priority substances in caged gammarids as a tool for chemical monitoring of waterways. However, this approach provides information on contaminant bioavailability without accounting for their toxic effects on organisms. To address this limitation, we developed a mechanistic toxicodynamic (TD) model based on the GUTS (General Unified Threshold Model of Survival) formalism, which links bioaccumulated concentrations to lethal toxicity in adult and embryonic gammarids.

Our work focused on two metals classified as priority pollutants: zinc, an essential metal, and nickel, a non-essential metal. Laboratory experiments were conducted to generate the necessary data for model calibration. A toxicity experiment assessed adult and embryo survival rates over 21 days under various exposure concentrations. In parallel, a bioaccumulation experiment measured the dynamics of metal uptake and elimination in gammarids, allowing the calibration of a one-compartment toxicokinetic (TK) model.

The TD model was then adapted to incorporate bioaccumulated concentrations as the primary input variable, as environmental monitoring primarily provides this type of data. Results showed that embryos

are more sensitive to nickel than adults, with faster toxic effects, whereas embryos are less sensitive to zinc but experience more rapid toxicity progression than adults. This work demonstrates the potential of integrating GUTS-based modeling into national biomonitoring frameworks to provide ecotoxicologically relevant insights. The model offers a robust approach to interpreting bioaccumulation data in terms of adult and embryonic toxicity, aiding in the assessment of metal impacts in aquatic ecosystems.

3.04.P-Th095 Cobalt Contamination Drives the Structure and the Co-occurrence Network of Freshwater Biofilms

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Cobalt (Co) is a key metal in the energy transition, which has led to an increase of its extraction and use over recent decades. Nevertheless, its impact on aquatic ecosystems is poorly understood. Biofilms are important biological components and indicators for river health. The present study thus examined Co impacts on the structure, interactions and functioning of prokaryotic and eukaryotic communities in growing biofilms.

Artificial substrata were placed in mesocosms (TotalEnergies facilities, Lacq, France) without (control) and with Co addition (0.1, 0.5 and 1 μ M). These substrata were then colonized by biofilms for 28 days. After the exposure period, a further 35 days without Co contamination was applied to simulate a recovery period. Water and biofilm were collected every 7 days during Co exposure. Water quality was analyzed, and biofilms were examined for Co bioaccumulation, structure and functions.

Cobalt bioaccumulation in growing biofilms was correlated with ambient free Co concentrations. Exposure at 0.5 and 1 μ M Co impacted beta-diversity of both prokaryotic and microeukaryotic communities from the earliest stages of biofilms colonization. Proportions of major prokaryotic and microeukaryotic taxa varied, highlighting sensitive and resistant taxa in both populations. Functional predictions revealed that Co exposure also influenced processes of biofilm formation (primary production and cellular processes). Finally, co-occurrence analyses indicated a reorganization of networks structure and dynamics in response to Co contamination. A loss of connectivity within microbial communities from exposed biofilms was highlighted and associated with a decreasing number and diversity of keystone OTUs. However, networks were denser with a concentration of interactions around the last central nodes, which were essential in the stress response and continued ecological function of the biofilms. Our results highlighted a new approach to assess Co impacts on biofilms considering co-occurrence patterns between microorganisms, in addition to analyses of taxonomic or functional profiles.

3.04.P-Th099 Pristine Ultramafic Waters Exhibit Baseline Chronic Ecotoxicity to *Daphnia magna* *Davide A. L. Vignati¹, Carole Cossu-Leguille², Elsa Salles¹, Celine Simon², Maximilien Beuret², Clement Bojic¹, Vincent Normant¹, Yann Sivry³, Thi Tuyen Nguyen³, Alexis Groleau³, Remi Marsac³, Yulia Erban-Kochergina⁴ and Pavel Kram⁴, (1)Universite de Lorraine, CNRS, LIEC, France, (2)Université de Lorraine, CNRS, LIEC, France, (3)Universite de Paris, CNRS, IPGP, France, (4)Czech Geological Survey, Division of Geochemistry and Laboratories, Czech Republic*

Ultramafic waters (UW) have naturally high concentrations of nickel (Ni), chromium (Cr) and Cobalt (Co); their relative abundances depending on the geology and mineralogy of parent rocks. Even in the absence of anthropogenic impacts, concentrations of these elements can therefore exceed the corresponding ecotoxicological standards or guidelines values for freshwaters. Because ultramafic rocks are mined for Ni, a key element for the energy transition, understanding the baseline ecotoxicity of pristine UW can assist responsible mining practices.

Water samples were collected in a pristine ultramafic catchment (Pluhuv Bor creek PLB, Czech Republic) far from anthropogenic inputs of trace elements except for atmospheric deposition. Nickel and chromium speciation was studied by filtration (0.22 μ m), ultrafiltration (3 kDa) and diffusive gradients in thin films (DGTs). Previous research showed that Co levels in PLB waters were below thresholds of ecotoxicological concern. Ecotoxicity testing was performed according to OECD guideline 211 (*Daphnia magna* reproduction test) with survival, growth and reproduction as endpoints. Heartbeat rate and swimming behavior were also evaluated at the end of the test.

Filterable (147 175 μ g/L) and ultrafilterable (35 40 μ g/L) Ni concentrations largely exceeded the European EQS of 4 μ g/L bioavailable Ni. Bioavailability corrected Ni concentrations (15 18 μ g/L) were also above the EQS and were similar for filtered and ultrafiltered waters. DTG-labile Ni concentrations (24 29 μ g/L) compared favourably with ultrafilterable ones. Total filterable Cr concentrations (21.4 22.5 μ g/L) were above the corresponding WHO guidelines: 4 μ g/L for Cr(VI) and 10 μ g/L for Cr(III).

However, more than 85% of total filterable Cr was associated with colloids and the corresponding ultrafilterable concentrations (2.8–3.2 µg/L) were below the most stringent guideline for Cr(VI). DGT labile Cr(VI) concentrations (1.4–1.8 µg/L) were also below the Cr(VI) guideline, while DGT-labile Cr(III) was below detection limit. Exposure to PLB waters did not affect survival and heartbeat rates of exposed daphnids. On the other hand, growth and reproduction were reduced in some samples and changes in swimming behaviour were observed in all samples. Although PLB waters caused sublethal responses in *D. magna*, further research is needed to clarify the exact causal relationships between Ni and Cr levels vs observed biological responses in *D. magna*.

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3.04.P-Th107 Updating the Bioavailability-Based Environmental Quality Standard Derivation Approach for Compliance Assessment of Zn in Freshwaters

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Many countries list Zn as a River Basin Specific Pollutant under the EU Water Framework Directive (WFD). Given the dependency of the toxicity of Zn on local water body conditions, it is essential that the derivation of Environmental Quality Standards (EQS) for Zn are bioavailability-based. In the present study, the existing bioavailability-based EQS derivation approach, which has been widely used and accepted for more than a decade; was updated by I) revising the existing ecotoxicity dataset, and updating it with recent high-quality ecotoxicity data for freshwater organisms, including species that were previously not considered and II) optimising the bioavailability models in terms of model structure and speciation software. The updated EQS derivation approach was then used to derive a bioavailable EQS for Europe, which can serve as a first-tier screening level in compliance assessments.

The updated chronic Zn freshwater ecotoxicity dataset combines high-quality data retained from the existing Zn freshwater toxicity datasets with high-quality data identified in a recent literature screening. The updated database now contains data covering 40 species and covers the minimum data requirements for species sensitivity distribution-extrapolation set out in the relevant guidance of the WFD. The existing chronic Zn bioavailability models were revised by optimizing the model structure and using the most recent version of WHAM as the underlying speciation software. Within the validation of the updated models with independent data for nine aquatic species: EC10 were generally predicted within 2-fold error for model-species and within 3-fold error for non-model species with few exceptions. Hence, the updated bioavailability models can be reliably used to accurately predict Zn toxicity across different species. The updated ecotoxicity dataset and the updated chronic bioavailability models were combined with species sensitivity distribution techniques resulting in the updated EQS derivation approach for Zn, which allows the derivation of a site-specific environmental threshold for Zn. The updated EQS derivation methodology has been used as a basis to derive a robust bioavailable EQS for Zn in European freshwaters. The bioavailable EQS in combination with the updated bioavailability normalisation approach will improve the environmental relevance of compliance assessment for Zn under the WFD.

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3.04.B Scientific Advancements in the Fate and Toxicity of Metals: Data, Models, Tools, and their Application in Environmental Regulations

3.04.B.T-01 Source-Oriented Risks of Heavy Metals and their Effects on Resistance Genes in Natural Biofilms

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Heavy metal (HM) introduction from various land-use patterns can be a major source of metal resistance genes (MRGs) entering river environments. This influx can trigger the occurrence of other resistomes, such as antibiotic resistance genes (ARGs), by improving co-resistant conjugative transfer. Biofilms, which form at water solid interfaces, could serve as potential hotspots for HMs and resistance genes.

However, the enrichment of HMs from various sources within biofilms and their effect on resistomes remain undocumented. This study aims to investigate the physicochemical properties of biofilm samples collected from the Heihui River, a tributary of the Lancang River, and to analyze the concentrations of nine HMs (As, Cd, Co, Cr, Cu, Ni, Pb, V, and Zn) within these biofilms. The 16S rRNA gene and metagenomic high-throughput sequencing techniques were integrated to uncover the association between HM accumulation levels in biofilms and ecological and health risks, considering the presence of two resistance genes. Natural sources (Co, Cr), industrial (As, Cu, V), agricultural (Cd, Ni), and transportation activities (Pb, Zn) markedly contributed to HM presence within biofilms, with industrial activities posing higher noncarcinogenic and carcinogenic risks than other sources. The network correlation analyses revealed higher levels of ARG MRG coexistence in biofilms, with the ecological and health risk index of HMs in biofilms closely associated with the abundance of both resistance genes. Furthermore, the biofilm As concentration markedly affected the abundance and expression of ARGs and MRGs, with elevated As levels within biofilms significantly and positively influencing all four functional categories of MRGs. Water pH also indirectly impacted these functional types by modulating the ionic form of HMs within the biofilm matrix. Our findings underscore the significance of integrating biofilms into environmental management practices and standards for assessing environmental quality.

3.04.B.T-02 Metal Toxicity From Hydrothermal Vent Sediments and Its Impact on Deep-Sea Mining

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Deep-sea mining, aimed at extracting metal-rich sulfides containing manganese, iron, and nickel from hydrothermal vents along mid-ocean ridges, presents significant environmental risks despite its economic allure. These sulfides are formed when hot fluids from vents mix with cold seawater, causing metals to precipitate and accumulate on the seabed. From March to April 2023, the Korea Institute of Ocean Science and Technology (KIOST) conducted the MIRAE expedition in the Indian Ocean's mid-ocean ridge. Utilizing the R/V ISABU research vessel and a remotely operated vehicle (ROV), sediment and rock samples were collected from six sites, particularly focusing on black smokers and hydrothermal vents known for their metal concentrations. Toxicity tests with amphipods (*Tiburonella viscana*) and mussel embryos (*Perna perna*) revealed severe environmental hazards. Sediment toxicity tests caused 100% mortality in amphipods, indicating extreme toxicity. Aqueous toxicity tests with elutriates, solubilized extracts, and leachates exhibited variable toxic effects, with some samples showing significant impacts at low concentrations (LOEC as low as 12.5%). Using the Toxicity Identification and Evaluation (TIE) approach, EDTA and C18 filtration reduced toxicity, pointing to metals and organic contaminants as the primary toxic agents. Observed effects included high lethality in test organisms and developmental abnormalities in mussel embryos. These findings underscore the ecological risks of deep-sea mining, such as harm to fragile ecosystems, water quality degradation, and long-term environmental damage. The study highlights the urgent need for robust monitoring, stringent regulations, and thorough environmental impact assessments to reconcile the economic potential of seabed mining with the preservation of deep-sea ecosystems.

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3.04.B.T-03 Evaluating the Impacts of Nickel on Arctic Freshwater Biota, from Crustaceans to Fish **Connor B. Stewart¹, Emily Rogevich Garman², Christian Schlekut², Elizabeth Middleton², Daniel Alessi³, Anne Cremazy⁴ and Tamzin Blewett⁵, (1)Biological Sciences, University of Alberta, Edmonton, Canada, (2)NiPERA Inc, United States, (3)University of Alberta, Canada, (4)INRS, Canada, (5)Biological Sciences, University of Alberta, Canada**

Nickel (Ni) is an essential trace metal of significant economic importance, particularly in the context of transitioning to green energy. Over the past decade, global demand for Ni extraction has surged, but despite this there is limited understanding of the environmental impacts associated with this increased demand. Arctic environments are at risk of such contamination, with significant Ni mining occurring in Northern zones. Nickel toxicity is dictated by its bioavailability to organisms in the receiving environment, with many Arctic freshwaters predicted to have high Ni bioavailability due to their abiotic composition (hardness, pH, DOC). Additionally, the unique physiology of Arctic organisms may alter their responses to Ni exposure, making extrapolation of results from temperate model organisms less feasible. The work presented here focused on two crucial Arctic relevant species: Arctic char (*Salvelinus*

alpinus), an important salmonid species at the top of many Arctic aquatic food webs, as well as *Daphnia pulex*, a crucial invertebrate and primary consumer. Juvenile arctic char were chronically exposed to a range of environmentally relevant Ni concentrations, finding significant growth rate reductions with a 60-day EC20 (Ni concentration causing a 20% reduction in growth rate) of 436 µg/L and significant mortality at higher concentrations, with a 28-day LC10 (Ni concentration killing 10% of exposed organisms) of only 1,111 µg/L, a concentration well within the range of Ni observed near Arctic mines. Additionally, relevant Ni concentrations caused significant alterations to the gill structure of exposed fish. Chronic Ni exposure to *D. pulex* showed significant reductions in survival and reproduction at concentrations orders below those observed downstream of mining operations in the Arctic in waters mimicking the ionic composition of Arctic freshwater systems. Further *D. pulex* experiments are currently underway to better characterize the sublethal impacts of Ni in Arctic relevant waters, with a particular focus on the toxicity modifying effects of pH in these waters. Taken together, these results provide insight into the sensitivities of Arctic relevant biota to realistic Ni exposures and help characterize the risks posed to unique Arctic ecosystems.

3.04.B.T-04 Are Physiological Responses to Exposure to an Urban Trace Metal Elements Mixture Sex-Dependent in Zebra Finches?

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Trace metal elements and metalloids (TME) are known to affect birds physiology, like increasing physiological stress, inducing oxidative stress, lowering body mass or shortening telomere. However, little is known on the way the different sexes respond to TME contamination. Previous studies have shown sex differences in contamination which could be attributed to physiological or behavioural effects. As female and male physiology are different, we can expect TME to affect their physiology differently. The aim of this work is to investigate for evidences in sex-dependent physiological adverse effect in a passerine bird model in response to exposure to an urban relevant TME mixture. To do this, 40 zebra finches (*Taeniopygia guttata*) pairs were exposed through their drinking water for 60 days to a urban relevant TME mixture (350 mg.L⁻¹ Zinc, 60 mg.L⁻¹ Copper, 2 mg.L⁻¹ Nickel, 0.6 mg.L⁻¹ Cadmium, 10 mg.L⁻¹ Lead and 2 mg.L⁻¹ Arsenic). We focused on sublethal and non-invasive cues: Individual contamination was followed using feathers TME concentration when physiological stress was monitored using heterocytes / lymphocyte ratio combined with avian stress hormone (corticosterone) feathers concentration. We used hematocrit as a general blood marker. As oxidative stress markers, we used protein carboxylation when catalase and superoxide dismutase activities and plasma carotenoid content were used as markers of oxidative defenses. The glutathion and glutathion peroxydase activities were used as detoxification marker. We hypothesised that females and males would respond differently to all of these markers, as contamination seems to vary between male and female. Most of studies in controlled environment only focussed on male responses to TME or reproduction outcome without looking at female physiology. This work should enable a wider overview of the direct effects of TME on birds.

3.04.B.T-05 Aquatic Ecotoxicity of Environmentally Realistic Metal-Organic Mixtures to the Algae *Raphidocelis subcapitata*

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Current environmental risk assessment frameworks predominantly rely on a substance-by-substance approach. This could lead to an underestimation of the environmental risk, especially if unintended combined effects could arise even when the components of a mixture are present at concentrations below their safe levels.

This study investigates the toxicity of metal-organic mixtures by conducting 72-hour growth inhibition tests with the algae *Raphidocelis subcapitata* (OECD 201). Binary mixture tests were designed to evaluate two hypotheses: (1) that independent action (IA) provides a more accurate predictive model than concentration addition (CA) for metal-organic mixtures, and (2) that organic chemicals present at no-effect concentrations do not influence metal toxicity, and vice versa. If the second hypothesis holds, it would suggest that metals and organic chemicals could be assessed independently in regulatory mixture frameworks.

Organic substances frequently co-occurring with metals in European freshwater were prioritized using monitoring data, resulting in the selection of ten metal-organic combinations. Each experiment involved testing the individual components and their binary mixtures simultaneously, following three test rays: (1) an equitoxic ray based on EC10 values, (2) a design where the organic concentration was held constant at half its EC10, while the metal concentration was increased, and (3) a reverse design in which the metal concentration was kept at EC10/2 while the organic one increased. The results of the first three mixtures (diuron-Cu, terbuthylazine-Cd and terbuthylazine-Zn) are presented. Diuron and terbuthylazine are photosystem II inhibiting herbicides.

On average, CA was slightly more accurate than IA, with no statistically significant trend toward synergistic or antagonistic interactions relative to either model. The comparisons between the metal or organic tested individually and in the presence of low concentrations of the other revealed statistically significant effects in four out of the six cases. In three cases, the toxicity was increased by the presence of the other, while in one case it was inhibited. This suggests that PSII-inhibiting herbicides and metals should not be assessed independently in regulatory frameworks. Further testing with a broader range of metals and organics with diverse modes of action is planned to determine whether this conclusion can be generalized to the entire range of organic contaminants.

3.04.P Scientific Advancements in the Fate and Toxicity of Metals: Data, Models, Tools, and Their Application in Environmental Regulations

3.04.P-Th081 Levels of Trace Elements in Teas and Herbal Infusions Available on the Portuguese and French Markets

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Teas and herbal infusions (THI) are widely consumed for their recognized health benefits. However, bagged teas often contain a mixture of leaves, dust, and fannings, which can result in a higher content of trace elements (TE). TE dissolve in hot water and transfer from THI bags to the beverage. For instance, the accumulation of aluminum (Al) in tea infusions has been associated with Alzheimer's disease. Therefore, the imbalance of TE, mainly linked to anthropogenic sources, raises concerns about food safety and human health. This study aimed to determine the TE concentrations in THIs available on the Portuguese and French marketplace. THI infusions were prepared from Portuguese commercial products (n = 23) and from French commercial products (n = 9) and analysed by inductively coupled plasma mass spectrometry (ICP-MS) using an iCAP Q instrument. The TE content included Al, chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), As (arsenic), rubidium (Rb), strontium (Sr), cadmium (Cd), and mercury (Pb). Statistical data analysis was performed using jamovi 2.3.26. The TE profiles of Portuguese and French teas were similar, with most samples showing the order: Al>Mn>Rb> Zn>Sr>Cu> Cr>Co> Pb>As>Sn> Cd> Hg. Overall, Portuguese THIs had lower TE content than French samples, especially Cr, Al, and Rb. Average concentrations for Portuguese samples were 5.15 µg/L for Cr, 5856.24 µg/L for Al, and 496.39 µg/L for Rb, compared to 16.22 µg/L, 12927.04 µg/L, and 105.16 µg/L respectively for French samples. The higher TE content in French THI samples may be due to their composition, as French THI products often include flavors and blends, while Portuguese samples typically consist of a single type of tea or herb. However, tap water can introduce additional elements into THI. This study highlights the variability in trace element concentrations between Portuguese and French THIs, emphasizing the influence of product composition and potential external sources like tap water, which together underscore the need for monitoring and managing trace element exposure to ensure food safety and protect consumer health.

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3.04.P-Th082 Evaluation of Cadmium and Lead Contamination in Wild Dandelion (*Taraxacum officinale*) from Croatia: Implications for Food Safety and Public Health

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Dandelion (*Taraxacum officinale*) often considered a weed is a highly versatile and beneficial plant that has been used in food, medicine, and as a natural remedy for centuries.

Soil pollution by potentially toxic elements is of concern since they may transfer to the edible parts of the food, especially when collecting wild plants from not cultivated, and thus not controlled soil. As examples, cadmium and lead are heavy metals that can accumulate in the body over time, leading to a range of adverse health outcomes.

The European Food Safety Authority (EFSA) stipulated maximum mass fractions of certain metals in food stuff, e.g., 0.05 mg/kg and 0.1 mg/kg for cadmium (Cd) and lead (Pb), respectively. Cadmium is primarily absorbed in the gastrointestinal tract when ingested through food. It is then distributed throughout the body, where it binds to proteins and accumulates in various tissues, particularly the kidneys, liver, and bones. Lead is absorbed into the body through the gastrointestinal tract and is distributed to the bloodstream, where it can accumulate in various tissues, including the bones, brain, and kidneys.

Dandelion and soil samples were collected at 19 different places in Croatia, divided into different parts (root, stem, leaf, flower); thus, generating a total of 95 samples. The plant parts were washed with diluted nitric acid, dried at 105 °C, and ground for homogenisation prior to acidic microwave assisted digestion. The quantification of selected potentially toxic elements in the clear digest solutions was carried out using inductively coupled plasma mass spectrometry (ICP-MS). Soil samples were air-dried and pulverized using an agate mortar prior to digestion and analysis. All plant samples had Pb mass fractions above the maximum value, the results ranging from 0.116 mg/kg to 2.39 mg/kg for the flowers, 0.272 mg/kg to 13.9 mg/kg for the leaves, 0.217 mg/kg to 3.56 mg/kg for the stem, and from 0.745 mg/kg to 40.4 mg/kg for the roots. Regarding Cd, all values except for flower and stem in two plants and four flowers exceeded the maximum mass fraction. On the contrast only five soils samples exceeded the World Health Organization (WHO) permissible limit of heavy metals in soil for Cd and only one for Pb.

The elevated levels of Cd and Pb in wild edible plants may pose a public health concern due to the exceedance of recommended limit values. In conclusion of this study, the present dandelion samples cannot be recommended for human consumption.

3.04.P-Th083 Assessing Heavy Metal Contamination from Shooting Ranges: A Case Study of the Munkatorp Shooting Range in Örebro, Sweden

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Conifer needles have been extensively studied worldwide, particularly in regions impacted by heavy metal pollution from traffic and industrial activities. These studies focus on evaluating the potential of pine needles as bioindicators of environmental contamination, due to their year-round presence. In addition to industrial pollution, shooting ranges are another significant source of heavy metal contamination.

Shooting ranges provide a controlled environment for firearm practice and training, yet the environmental impacts of these activities are often overlooked. The discharge of lead and other heavy metals from ammunition poses a serious threat to ecosystems and public health. Lead is a major contaminant, as bullets are typically composed of lead or contain lead-based components. When fired, these bullets release lead particles into the environment, contaminating soil and water. Over time, the accumulation of lead in the ecosystem can have harmful effects on plants, wildlife, and human health.

One such area of concern is the Munkatorp Shooting Range in Örebro, Sweden, which has been identified as a site of significant heavy metal pollution. Although there are plans for decontamination, no substantial actions have been taken to address the contamination so far. Within the designated area, four trees were selected for sampling: two Norway spruces (*Picea abies*) and two Scots pines (*Pinus sylvestris*). Needle samples were carefully collected and stored in plastic bags, then washed, dried, and ground into a fine powder for subsequent digestion and analysis. The metal content was quantified using inductively coupled

plasma mass spectrometry (ICP-MS) after acidic digestion. The study focused not only on metals commonly associated with ammunition, specifically manganese, iron, nickel, copper, zinc, molybdenum, antimony, and lead, but also on essential elements for plants to better understand the environmental impact of the pollution in this area. Furthermore, the needles underwent analysis via Raman Spectroscopy to get information on the metabolic state of the plant material, which provides deeper insight on the soil conditions on the growing site of the conifers. The analysis of conifer needles revealed no significant contamination attributable to the shooting range. Instead, it highlighted species-specific variations in metal contents, but within the ranges reported in literature. Species-specific differences were also found using Raman Spectroscopy.

3.04.P-Th084 Analysis of the Otolith Bone for Tracing Environmental Metal Contamination in Bluegill (*Lepomis macrochirus*) and Green Sunfish (*Lepomis cyanellus*)

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Otoliths, or "ear stones," are calcareous structures in fish that aid in balance and sound detection, and their chemical composition can serve as bioindicators for aquatic environments. This study uses otolith analysis to examine the life history of metal exposure on fish from the Grand Lake and Tar Creek Superfund site in Oklahoma a historically lead- and zinc-mined area with extensive contamination. The mechanisms of metal deposition into otoliths and their link to contamination levels remain poorly understood, warranting investigation into how pollution at the Tar Creek Superfund site influences otolith biomineralization and records of historical exposure.

To evaluate whether otoliths can reflect the life history of metal exposure, *Lepomis* species (bluegill and green sunfish) were sampled from four sites along a contamination gradient: two reference sites (Grand Lake Honey Creek and Sycamore Creek) and two polluted sites (Tar Creek E40 and Miami, near the mine drainage). Dissolved metals concentrations in the water revealed a pronounced pollution gradient, with cadmium levels ranging from 0.10 µg/L at reference sites to over 12 µg/L at E40, and zinc levels exceeding 7,000 µg/L at E40 compared to under 2 µg/L at reference sites. Copper, lead, and iron were similarly elevated at polluted sites, reflecting persistent mining-derived contamination. Fish otoliths from toxic sites were notably lower in weight comparison (12.06 ± 3.50 mg vs. 18.03 ± 8.20 mg) but greater in length (0.477 ± 0.11 cm vs. 0.352 ± 0.09 cm) and width (0.178 ± 0.22 cm vs. 0.117 ± 0.03 cm), suggesting some effects on otolith biomineralization. Relative weight, a ratio of a fish's actual weight to its expected weight based on species and size, was lower at polluted sites (E40: 84.03/Miami: 79.42) than at reference sites (GLHC: 87.17/SYC: 84.82).

To further explore these findings, sagittal otoliths were dissected and analyzed for metal accumulation. One otolith was dissolved in nitric acid for trace metal analysis via ICP-MS, while the second will undergo laser ablation ICP-MS and X-ray fluorescence to examine spatial patterns of elemental deposition. This approach correlates environmental metal concentrations with temporal bioaccumulation in fish. By linking otolith chemistry to water quality, this study offers insights into contamination impacts on fish health and evaluates remediation efforts in the Grand Lake region.

3.04.P-Th085 Where's the Data? Updating the EU Physicochemical Freshwater Monitoring Database

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A number of metal Environmental Quality Standards (EQS) are based on the bioavailable fraction of a metal, and therefore, this needs to be accounted for during an EQS compliance assessment. However, when the guidance for using bioavailability was set there was a paucity of samples with parameters required for bioavailability correction, many existing databases were only snapshots of discrete timeframes, or of uncertain quality, which limited the ability to perform such an assessment. Launched in 2020, the EU Physicochemical Database Tool aimed to remedy this by compiling data from EU Member States (EU MS). The database is designed to assist practitioners in obtaining data for European freshwaters to allow for efficient metal EQS compliance assessments, particularly by targeting data on pH, dissolved organic carbon and calcium concentrations as well as metal exposures.

Databases, however, should not be static as data is constantly generated by environment agencies and international commissions, and the data usages rights change. Therefore, an update of the Database has

been performed. This involved contacting all EU MS, as well as select non-EU MS requesting data for a defined list of parameters (including the parameters required for bioavailability normalisation), and also covering additional parameters that were not included in the original data collection exercise, for example silver, cobalt and magnesium.

This exercise has resulted in the inclusion of 36 new datasets, containing data from 33 European countries, encompassing over 2,300,000 additional samples from more than 84,000 sampling sites. This is a significant increase from the original assessment that resulted in ~1,000,000 samples being collected from ~26,000 sites. The new data includes samples from countries that were previously available but could not be publicly shared, countries where bioavailability parameters were lacking (Portugal), and countries that were previously unavailable (Cyprus). Additionally, the spatial distribution has also increased for some countries where previously only limited data was available (e.g., Germany). All datasets collected have also been assessed using CREED for reliability and relevance for inclusion within the EU Physicochemical Database.

This poster will highlight the additional data collected, particularly the expansion in the number of parameters, the increase in spatial coverage, and general findings of the reliability and relevance assessment.

3.04.P-Th086 Simplified and Now User-Friendly! Updating Bio-Met for Better Risk Assessment

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A tiered approach under the European Water Framework Directive has been developed for implementing bioavailability-based Environmental Quality Standards (EQS) for metals. Tier 2 of the approach prescribes the use of simplified tools, often based on the full and complex biotic ligand models (BLMs), to account for bioavailability. Simplified tools are designed to be less data intensive and easier to use in routine regulatory activities. bio-met is one such simplified tool that only requires pH, dissolved organic carbon and calcium concentrations to assess the potential freshwater risks and compliance with EQSbioavailable for cobalt, copper, nickel, lead and zinc. While Tier 2 provides an opportunity for the use of simplified tools it has certainly been the case in the past that simplified tools have not always been simple to use.

Feedback has been received from a number of avenues, including workshops and directly from users, regarding potential improvements to bio-met from both scientific and usability perspectives. In bio-met version 5.1, the extraction of the calculated results from the tool is difficult, and the guidance associated with results is unclear and hard to review. Additionally, if only hardness was available, a separate step converting to calcium was required prior to processing in the tool. The updates to bio-met presented in this poster focus on the usability updates applied to the tool.

A functionality has been added so that the metal(s) of interest to be assessed can be selected to focus the outputs to the user's requirements and improve processing speed. Additionally, a function to export results has been added to make reviewing of outputs simpler and clearer. All guidance, and the front-page, have been reviewed and updated with additional context while also being clear and concise. Furthermore, the reliability of results produced for samples where a parameter is outside of the model applicability domain, compared to the full BLMs outputs, has been incorporated in the updated tool. Ultimately, this means that results will only be produced if one parameter is outside of the applicability domain and the full BLM indicates the result is not likely to be under-conservative.

These updates have been designed to encourage greater use of tool, to better implement the tiered approach, and to assist in producing high-quality evidence-driven conclusions when evaluating the potential risk of metals in European waters.

3.04.P-Th087 Determination of Gallium (III) and Tin (II) Free Concentrations in Aqueous Media with the Electroanalytical Technique AGNES (Absence of Gradients and Nernstian Equilibrium Stripping)

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The use of gallium and tin has increased recently, especially due to their technological applications. The

potential effects of the release of these elements to the environment calls for the study of their bioavailability. The essential tenet of ecotoxicological models such as the Free Ion Activity Model (FIAM) or the Biotic Ligand Model (BLM) is that toxicity is strongly correlated to the concentration of the free metal ion. So, the development of techniques to measure these concentrations in various aqueous media is needed. The electroanalytical techniques AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) has been designed to measure free metal ion concentrations. Typical analytes were Zn(II), Cd(II) and Pb(II) in a variety of matrices ranging from estuarine water to soil extracts, from ZnO nanoparticle dispersions to growth media, from wine to streams in the Pyrenees, etc. More recent analytes were Sb(OH)₃ and In(III). For indium, attomole per litre concentrations were reached in dispersions of In₂O₃ nanoparticles in synthetic seawater. In this presentation, the implementation of AGNES to determine Ga(III) and Sn(II) will be reported. In both cases, their strong hydrolysis has led -as a first step- to the analysis in acidic conditions (up to pH 4). The electrodic irreversibility of the couple Ga⁰/Ga³⁺ on the mercury electrode implies longer deposition times than when dealing with the typical divalent cations. In the case of Sn(II), an added challenge is its easy oxidation to Sn(IV). In both cases, the free metal ion concentrations determined with AGNES are checked against those predicted by the speciation code VMINTEQ for Ga³⁺ complexation with phthalate and Sn²⁺ complexation with EDTA.

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3.04.P-Th088 Possibilities and Challenges in Setting Scientifically Defensible Discharge Limits for Aluminium in the UK

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Aluminium is naturally occurring and abundant in the environment due to its presence in many minerals. It also has several uses including in cleaning and personal care products, industrial processes, and as a flocculant to remove nutrients from the effluent of wastewater treatment works, often in the form of aluminium sulphate (alum). Concentrations of residual aluminium may remain in wastewater effluents post treatment, resulting in potential releases to receiving waters. The assessment of potential aquatic risks from aluminium in freshwaters is complicated by the range of factors that can influence its speciation and ecotoxicity in the water environment. This means the speciation and ecotoxicity of aluminium is not straight-forward to predict or model. Furthermore, the lack of a sufficiently practicable measurement approach that can readily distinguish between potentially toxic and non-toxic forms of aluminium from natural background sources, such as aluminosilicate minerals present in suspended particulate matter is challenging. This complexity has hindered the progress on the development of an evidence-driven limit values and standards for aluminium. This presentation considers the possibilities for the development of robust discharge consents for aluminium in freshwaters that can take account of the differences in sensitivity to aluminium between different sites.

There is a good body of evidence to support the need to take account of both the bioavailability of aluminium and its tendency to form precipitates under certain conditions in setting a standard. However, a lack of sufficient validation information for the available bioavailability models has prevented the setting of an EQS in the UK during previous reviews. This project evaluates whether field evidence based on invertebrate communities can be used to demonstrate the protectiveness of the bioavailability models and any standards that they are used as the basis for. Recommendations are made regarding the most appropriate method for setting discharge consents for aluminium containing effluents from wastewater treatment plants.

3.04.P-Th089 Free Ion Determinations to Support Silver Bioavailability Model Development

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Development of bioavailability models for metals in aquatic systems requires computation of metal speciation in the exposure medium of the ecotoxicological experiments used for model calibration. Almost always, the most important speciation model output is the activity of the free metal ion since this is almost invariably a key chemical variable within the bioavailability model. Such computations may be carried out using a speciation model, such as WHAM7 or Visual MINTEQ, if it is parameterised for the reactions of the metal of interest with solution ligands. Ligands include dissolved organic matter (DOM), if exposure waters containing DOM are to be used for model development. While the availability of a parameterised speciation model is a prerequisite for bioavailability model development, model parameters, particularly those for interaction with DOM, may not be extensively validated. Furthermore, there may be a need to gain further knowledge on the influence of variability in metal DOM binding strength on the bioavailability of the metal.

Analytical determination of free metal ion activities in the exposure waters can provide invaluable data to address both these issues. Here we present the results of ongoing research to set up and apply a system for determination of the free silver ion activity in test solutions and ecotoxicity test media for algae (*Species*) and *Daphnia magna*. This research is a component of a project to develop and apply chronic bioavailability models for silver to aquatic organisms. To achieve the widest possible analytical window for silver ions, we have adapted the flow cell approach used by Tait and co workers (doi.org/10.1071/EN14190) for silver, and have tested electrode linearity both in simple solutions and in solutions containing well defined synthetic organic ligands. We will present measurements of free silver ion activities in exposure media, including media containing natural DOM, over the ranges of the dissolved silver used in toxicity testing, and provide comparisons to free ion predictions made by the WHAM7 model.

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3.04.P-Th090 Development of an Approach to Account for Bioavailability in the Assessment of Potential Risks of Uranium Exposures to Freshwater Ecosystems

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"Water chemistry has been shown to influence the speciation and bioavailability of metals in freshwater ecosystems. Relationships have been developed to estimate the bioavailability and subsequent ecotoxicity of metals from water chemistry parameters, such as pH, dissolved organic carbon (DOC) and hardness. These relationships, developed into models such as Biotic Ligand Models (BLMs) and Multiple Linear Regressions (MLRs), once successfully validated against ecotoxicity tests in field collected waters, have been adopted by regulatory authorities around the world to provide evidence-driven assessment of potential risks. Bioavailability-based approaches are available for many metals. However, despite the recognised importance of water chemistry conditions in determining potential ecotoxicity no models, beyond simple DOC corrections, have been developed for uranium. In addition to the obvious emission sources of uranium from the global nuclear industry and mining operations, uranium occurrence is widespread as a natural component of geological matrices and bedrocks. Here we provide an outline of the approach being taken in developing an understanding of uranium bioavailability, that can provide fit for purpose assessment of uranium ecological risks in freshwaters. The key tasks in our approach are:

3.04.P-Th091 Initial Outcomes in the Development of a Bioavailability-Based Approach to Risk Assessment of Uranium in Freshwaters: Data collection and likely bioavailability relationships

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Uranium may enter freshwater ecosystems through emissions from industrial activities such as mining or the generation of nuclear power. Importantly, a further source of uranium may also be from geogenic sources when underlying parent materials are disturbed by infrastructure developments or mining activities. It has long been established that water chemistry conditions may influence uranium speciation and ecotoxicity in freshwaters. However, a robust approach to account for uranium bioavailability is not yet available.

Here we describe the outcomes from the early stages in the development of an approach to account for bioavailability, that is both scientifically robust and can be practically applied by stakeholders and regulators.

A comprehensive search of the scientific literature was conducted to identify potentially relevant

ecotoxicity data for uranium. In addition, the results were cross-checked with uranium EQS dossiers published by the Netherlands and Canada. An ecotoxicity database was developed, containing nearly 400 individual datapoints covering a wide range of species and taxonomic groups. The data were assessed for their suitability for use in bioavailability modelling based on reported water chemistry parameters. Moderate quantities of chronic data representing a range of water chemistry conditions are available for several species, and efforts will be made to identify the key drivers of uranium toxicity from them. However, because these tests were not conducted with the intention of developing reliable bioavailability models for regulatory applications additional testing will be required to ensure that the models are both sufficiently reliable and cover the required range of water chemistry conditions. Various established modelling approaches (e.g., BLM, gBAM, MLR) will be taken to explain the relationship between water chemistry and uranium toxicity to aquatic organisms. Additionally, there are also more limited quantities of data available for several other species that provide an opportunity to validate the bioavailability models that are developed. The ranges of water chemistry conditions that the models need to be applicable to, and the extent to which the models developed can reliably predict uranium toxicity will also be addressed. Insights into the sensitivity of both Swedish and European surface waters to uranium toxicity will also be presented.

3.04.P-Th092 Delivering a New Framework for the Implementation of Bioavailability-Based Metal Guideline Values in Australia and New Zealand

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"A new framework for the derivation and implementation of bioavailability-based metal Default Guideline Values (DGVs) has been developed for Australia and New Zealand. The approach was formulated during a 2023 workshop held in Sydney and comprised of scientists and regulators from New Zealand and Australian states and territories. Specifically, the initial focus has been on copper, nickel, and zinc and has included key activities such as the:

3.04.P-Th093 Role of Medium Anionic Composition on Manganese and Zinc Cytotoxicity in Rainbow Trout Gill Cells

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Research has shown that the bioaccumulation of manganese (Mn) in animals can lead to neurodegenerative diseases such as Parkinsonism. Despite its potential dangers, limited data exists about the uptake mechanism and cytotoxicity of this essential metal and how the medium composition can affect bioavailability. Similarly, zinc (Zn) is another essential metal whose bioavailability and toxicity can be affected by the interaction with anions in the exposure medium. To evaluate the role of medium composition on the bioavailability and toxicity of Mn and Zn, we used a model of the rainbow trout gill cells, RTgill-W1 cell line. The RTgill-W1 cells were seeded in 24-well plates at 79,000 cells/cm², incubated for 48 hours, and exposed to each metal separately for 24 hours in three distinct media. The role of exposure medium composition was evaluated by manipulating anionic composition to produce these media: (1) L15/ex, a phosphate-buffered medium; (2) L15/P0, a HEPES-buffered medium; and (3) L15/HCO₃, a bicarbonate-buffered medium. A chemical equilibrium model, Visual MINTEQ, was used to determine the speciation of Mn and Zn in the different media. In the L15/ex medium, free Mn²⁺ was lowest at 79.199% but 92.567% in the L15/P0 medium, with the L15/HCO₃ medium containing a value in between. Uniquely, the L15/HCO₃ medium allowed the formation of the MnHCO₃⁺ and MnCO₃ (aq) species compared to L15/Ex and L15/P0. For Zn, only L15/HCO₃ and L15/Ex were analyzed. In L15/HCO₃, 4.075% ZnCO₃ (aq), 3.311% ZnHCO₃⁺, and 0.014% Zn(CO₃)₂-2 were present. In contrast, Zn in L15/Ex distinctly forms ZnHPO₄ (aq). After collecting this data, multiple endpoint viability assays measuring metabolic activity, membrane integrity, and lysosome integrity were used to determine metal cytotoxicity in RTgill-W1 cells. This approach allowed for the linkage between the role of extracellular metal speciation and intracellular toxicity. The results of these assays show that the Mn EC₅₀ value in L15/ HCO₃ is 213.7μM compared to 18,918μM in L15/ex. This is especially probing because no other divalent metal has produced such a drastic effect on the RTgill-W1 cells in the L15/HCO₃ medium. This finding possibly points to an unknown metal cotransporter using bicarbonate. In future research, the interactions between multiple metals in different media will be studied, which will hopefully help to fill knowledge gaps about metal toxicity.

3.04.P-Th094 Assessing the Oxidative Potential of Metals in Thoracic-Sized Fractions of Road Dust
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Mass measurements of airborne PM have informed our current understanding of the importance of respiratory exposures for human health. It is hypothesized that documented health impacts are mediated via the generation of reactive oxygen species (ROS) leading to oxidative stress. In line with this, attention has shifted to using chemical assays to measure the oxidative potential (OP) of airborne PM to supplement traditional mass measurements to obtain a more holistic understanding of risk. Road dust is a recognized important non-exhaust source of air pollution. This includes metal-bearing particles released from asphalt and tire and brake wear, which may catalyze the generation of reactive oxygen species (ROS). In contrast to studies of airborne PM, the OP of finer road dust fractions has yet to be examined. Given this, the need to characterize its OP to better understand the potential contribution of road dust to airborne pollutant exposures is highlighted.

This study aims to measure the OP of metals in thoracic-sized road dust (<10 μ m) collected in Toronto, Canada. To assess the OP contribution of soluble vs. insoluble elemental fractions, samples were first treated with Gamble's to simulate the neutral, interstitial conditions in the lung. Water was also used for comparison. OP was measured using the ascorbic acid (AA) and dithiothreitol (DTT) assays (referred to as OPAA and OPDTT). For this, incubated samples were placed in UV-transmissible 96-well plates and spiked with either AA and DDT solutions to achieve final concentrations of 200 μ M and 100 μ M, respectively. Absorbance was measured over specified time intervals at 265 nm for the AA assay and 412 nm (after adding Ellman's reagent) for the DDT assay.

Soluble fractions contributed most to total OP (i.e., 90 $\pm$ 10 % of total OPAA and 77 $\pm$ 8 % total OPDTT). Metals accounted for most of total OPAA and OPDTT measured, with an average of 84% and 65%, respectively. Of all the elements, Cu, Mn, and Fe were determined to be responsible for most of the OP determined with the two assays (i.e., 83% for OPAA and 60% of OPDTT). The insoluble OPAA and OPDTT fractions did not correlate with their soluble counterparts, suggesting that different chemical components (e.g., organics) drive the OP in soluble vs. insoluble fractions. This study highlights the potential for finer road dust fractions to contribute to the OP of respiratory concern. The importance of transition metals, especially Cu, Mn and Fe, are emphasized.

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3.04.P-Th095 Cobalt Contamination Drives the Structure and the Co-occurrence Network of Freshwater Biofilms

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Cobalt (Co) is a key metal in the energy transition, which has led to an increase of its extraction and use over recent decades. Nevertheless, its impact on aquatic ecosystems is poorly understood. Biofilms are important biological components and indicators for river health. The present study thus examined Co impacts on the structure, interactions and functioning of prokaryotic and eukaryotic communities in growing biofilms.

Artificial substrata were placed in mesocosms (TotalEnergies facilities, Lacq, France) without (control) and with Co addition (0.1, 0.5 and 1 μ M). These substrata were then colonized by biofilms for 28 days. After the exposure period, a further 35 days without Co contamination was applied to simulate a recovery period. Water and biofilm were collected every 7 days during Co exposure. Water quality was analyzed, and biofilms were examined for Co bioaccumulation, structure and functions.

Cobalt bioaccumulation in growing biofilms was correlated with ambient free Co concentrations. Exposure at 0.5 and 1 μ M Co impacted beta-diversity of both prokaryotic and microeukaryotic communities from the earliest stages of biofilms colonization. Proportions of major prokaryotic and microeukaryotic taxa varied, highlighting sensitive and resistant taxa in both populations. Functional predictions revealed that Co exposure also influenced processes of biofilm formation (primary production and cellular processes). Finally, co-occurrence analyses indicated a reorganization of networks structure and dynamics in response to Co contamination. A loss of connectivity within microbial communities from exposed biofilms was highlighted and associated with a decreasing number and diversity of keystone OTUs. However, networks were denser with a concentration of interactions around the last central nodes, which were essential in the stress response and continued ecological function of the biofilms. Our results

highlighted a new approach to assess Co impacts on biofilms considering co-occurrence patterns between microorganisms, in addition to analyses of taxonomic or functional profiles.

3.04.P-Th096 Algal Acute Toxicity of Silver Cyanide in Freshwater Environments

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Silver cyanide (AgCN) is a stable silver (Ag) complex that is poorly soluble in water. The primary application of AgCN is in silver plating and electroplating processes. Cyanide (CN⁻) forms stable complexes with precious metals, including silver, suggesting incomplete dissociation of dissolved complexes in aquatic environments, as suggested in peer-reviewed literature and speciation modelling. Experimental evidence is however lacking to conclusively demonstrate if this distinct speciation also results in a distinct aquatic toxicity of AgCN compared to its most ecotoxic constituent, i.e., ionic silver (Ag⁺).

Therefore, an experimental testing program is developed to investigate this research question. Algae are identified as the most sensitive test species towards Ag⁺. Therefore, as a first step, an algal toxicity test with *Raphidocelis subcapitata* is conducted to compare the toxicity of AgCN with a readily soluble silver compound (AgNO₃). An initial dose range finding study suggests more than 10-fold higher toxicity thresholds for AgCN compared to AgNO₃, when toxicity is expressed as a function of dissolved Ag. A subsequent main algae toxicity test is currently ongoing. If this main test confirms the lower toxicity of AgCN compared to AgNO₃, additional trophic levels (invertebrates and fish) will be tested to ultimately allow a substance specific hazard and risk assessment of AgCN under EU CLP and EU REACH regulations. The data of this testing program and their implications in an EU Regulatory context will be presented during the SETAC conference.

3.04.P-Th097 Response of *Scenedesmus acutus* (Chlorophyta) Microalgae to Metals Exposure

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Scenedesmus acutus Meyen 1829 is a microalga that was isolated from water samples collected in the Xochimilco canals, where it serves as food for copepods, cladocerans and rotifers. In this study, the toxic effect of the metals Cd, Cr, Cu, Hg, Mn, Ni, Pb, V and Zn, which are the elements found in concentrations greater than 50 mg/L in the aquatic systems of the Valley of Mexico, was determined on 4 biomarkers: biomass production, chlorophyll concentration, phenol production, and lipoperoxidation in microalgae. Bioassays lasting 72 hours were performed to determine the EC₅₀ and in tests with a sublethal concentration (CL₁₀), for 10 days, the effect on the biomarkers was evaluated. The toxicity of the metals based on the calculated EC₅₀ was (from highest to lowest toxicity): Cu > Cr > Hg > Zn > Cd > Ni > Mn > Pb > V. In the sublethal bioassays with Cd, Ni, and Pb, a decrease in chlorophyll levels was observed. In the tests with Cd, Cr, Ni, Pb, and Zn, an increase in the levels of phenols and carotenes was detected. A high degree of lipoperoxidation was observed in the bioassays with Cu, Mn, and Zn. Because the EC₅₀ values for the metals Cd, Cu, and Pb are lower than those established by NOM 001-Semarnat for discharges into aquatic systems, it is important to continue conducting research and monitoring to detect responses that indicate possible damage to the populations of this microalgae by the action of the discharges, in order to avoid irreversible deterioration of the populations in the medium and long term.

3.04.P-Th098 Nickel Ecotoxicity to *Raphidocelis subcapitata* in Standard ISO Medium vs. Ultramafic Waters

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Pristine ultramafic waters (PUW) usually have Ni concentrations in excess of environmental quality standard (EQS) even in the absence of anthropogenic influence. However, PUW are also rich in colloidal carrier phases (e.g., Fe-hydroxides and natural organic matter) that can mitigate Ni ecotoxicity by reducing its bioavailability.

PUW were collected from the Pluhuv Bor creek (PLB, a small catchment in the western Czech Republic) during snowmelt and tested for their possible ecotoxicity to a model alga in relation to their high Ni content (> 100 µg/L). Ecotoxicity testing was carried using the freshwater alga *Raphidocelis subcapitata* and following the ISO norm 8692. Preliminary work established a Ni 72h-EC₅₀ of around 30 µg/L for algal growth in ISO medium; a value 3- to 5-fold lower than concentrations measured in PLB waters (114

164 µg/L filterable Ni; 0.22 µm). However, PLB waters did not reduce algal biomass compared with control exposures. Characterization of Ni speciation using filtration/ultrafiltration showed that 70–80% of filterable Ni was associated with colloids, resulting in ultrafilterable Ni concentrations (< 3 kDa) of 30–37 µg/L. Bioavailable Ni concentrations, estimated with a user-friendly BLM tool, were 12–17 and 8–13 µg/L in filtered and ultrafiltered waters, respectively; these values being close to the Ni 72h EC10 determined in standard ISO medium. Testing of PLB waters spiked with NiCl₂ (14–250 µg/L as Ni) did not result in appreciable ecotoxic effect, either. Modelled bioavailable Ni-concentrations in PLB samples spiked with 250 µg/L of soluble Ni were around 40 µg/L, i.e., above the EC50 value determined in ISO medium. Further research appears necessary to understand how the interplay between speciation and bioavailability affects the ecotoxic potential of Ni in the complex matrix of PUW.

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3.04.P-Th099 Pristine Ultramafic Waters Exhibit Baseline Chronic Ecotoxicity to *Daphnia magna*
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Ultramafic waters (UW) have naturally high concentrations of nickel (Ni), chromium (Cr) and Cobalt (Co); their relative abundances depending on the geology and mineralogy of parent rocks. Even in the absence of anthropogenic impacts, concentrations of these elements can therefore exceed the corresponding ecotoxicological standards or guidelines values for freshwaters. Because ultramafic rocks are mined for Ni, a key element for the energy transition, understanding the baseline ecotoxicity of pristine UW can assist responsible mining practices.

Water samples were collected in a pristine ultramafic catchment (Pluhuv Bor creek PLB, Czech Republic) far from anthropogenic inputs of trace elements except for atmospheric deposition. Nickel and chromium speciation was studied by filtration (0.22 µm), ultrafiltration (3 kDa) and diffusive gradients in thin films (DGTs). Previous research showed that Co levels in PLB waters were below thresholds of ecotoxicological concern. Ecotoxicity testing was performed according to OECD guideline 211 (*Daphnia magna* reproduction test) with survival, growth and reproduction as endpoints. Heartbeat rate and swimming behavior were also evaluated at the end of the test.

Filterable (147–175 µg/L) and ultrafilterable (35–40 µg/L) Ni concentrations largely exceeded the European EQS of 4 µg/L bioavailable Ni. Bioavailability corrected Ni concentrations (15–18 µg/L) were also above the EQS and were similar for filtered and ultrafiltered waters. DTG-labile Ni concentrations (24–29 µg/L) compared favourably with ultrafilterable ones. Total filterable Cr concentrations (21.4–22.5 µg/L) were above the corresponding WHO guidelines: 4 µg/L for Cr(VI) and 10 µg/L for Cr(III). However, more than 85% of total filterable Cr was associated with colloids and the corresponding ultrafilterable concentrations (2.8–3.2 µg/L) were below the most stringent guideline for Cr(VI). DGT labile Cr(VI) concentrations (1.4–1.8 µg/L) were also below the Cr(VI) guideline, while DGT-labile Cr(III) was below detection limit. Exposure to PLB waters did not affect survival and heartbeat rates of exposed daphnids. On the other hand, growth and reproduction were reduced in some samples and changes in swimming behaviour were observed in all samples. Although PLB waters caused sublethal responses in *D. magna*, further research is needed to clarify the exact causal relationships between Ni and Cr levels vs observed biological responses in *D. magna*.

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3.04.P-Th100 Cadmium Toxicity in *Daphnia magna* is Modified by Food Concentration
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Many metabolic and physiological processes in organisms are closely related to essential metals. However, non-essential metals lack identified functions in biological processes and can cause toxicity. Such is the case of cadmium (Cd), which is highly toxic and is released into the environment by natural sources and as a pollutant from anthropic activities. The demonstrated carcinogenic and teratogenic effects of Cd produce biological and ecological impacts in aquatic environments, affecting exposed organisms and the population structure of planktonic species, including both primary producers and filter-feeding consumers. This can lead to bioaccumulation processes that negatively affect higher trophic levels. In this context, the present study evaluated the toxic effect of cadmium on the cladoceran *Daphnia magna* clone F when fed with different concentrations of microalgae. For this, the median Inhibitory Concentration (IC50) 96 h for cadmium chloride (CdCl₂) was determined for the microalga *Ankistrodesmus falcatus*, as well as the median Lethal Concentration (LC50) 48 h for the cladoceran *D. magna*. A 21-day chronic bioassay was then conducted, exposing *D. magna* to 6 µg L⁻¹ of CdCl₂, feeding with 0.5x10⁷, 1x10⁷, and 2x10⁷ cells mL⁻¹ of *A. falcatus*, to evaluate the combined effects of both factors on reproductive responses. The concentrations of proteins, lipids, and carbohydrates in the cladoceran neonates were also quantified. The obtained values were IC50 = 108.02 µg L⁻¹ and LC50 = 20.63 µg L⁻¹ of CdCl₂. The reproduction of *D. magna* was affected, showing a decrease in fecundity, the number of broods, and an increase in the age at first reproduction. The macromolecule concentration in the offspring changed according to the amount of food provided and the concentration of cadmium. These findings highlight the importance of considering the interaction between metal toxicity and food availability in studying the impact on the biological and ecological responses of aquatic biota exposed to pollutants.

3.04.P-Th101 Gadolinium and Environmental Contamination: Effects Across Trophic Levels in Aquatic Ecosystems

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Rare earth elements (REEs) consist of fifteen lanthanides along with scandium (Sc) and yttrium (Y). Anthropogenic activities, such as medical waste, mining, and electronics production, are key sources of REEs contamination. Their widespread use has led to environmental contamination, particularly in aquatic and soil ecosystems. Indeed, aquatic organisms have been shown to suffer from malformations, reduced reproductive success, inhibited growth, and toxicity. Among the lanthanides, gadolinium (Gd) is commonly used as a paramagnetic contrast agent for MRI, with its presence detected in rivers, drinking water, and marine environments. However, detailed studies on gadolinium's toxic effects on organisms remain limited.

This study evaluates the ecotoxicological effects of Gd on model species at different trophic levels. Ecotoxicological tests were conducted on bacteria (*Aliivibrio fischeri*, bioluminescence inhibition), algae (*Phaeodactylum tricornutum* and *Raphidocelis subcapitata*, growth inhibition), crustaceans (*Daphnia magna*, mortality), and echinoderms (*Paracentrotus lividus*, spermioxicity and embryotoxicity). The tested gadolinium (Gd₂O₃) concentrations were environmentally relevant: 0 µg/L, 0.1 µg/L, 1 µg/L, 10 µg/L, and 100 µg/L.

Results indicate that gadolinium's toxicity increases with concentration. *Aliivibrio fischeri* showed biostimulation at low concentrations but up to 98.7% bioluminescence inhibition at 100 µg/L. *Phaeodactylum tricornutum* and *Raphidocelis subcapitata* experienced growth inhibition up to 97% and 96.2% at 100 µg/L. *Daphnia magna* survival remained stable up to 1 µg/L, dropping to 75% at 100 µg/L. *Paracentrotus lividus* displayed a 70.7% reduction in embryonic development at 100 µg/L. These findings show that gadolinium's toxicity is dose-dependent, with species varying in sensitivity, highlighting the ecological risks of Gd contamination and the need for further research.

3.04.P-Th102 Comparison of Toxicity and Bioaccumulation of Platinum Metals for Organisms of Different Trophic Levels

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The growing use of platinum group metals (PGM) in transport and industry has increased the content of these metals in various components of the environment, especially in the aquatic ecosystem. PGM undergo changes in the environment, become bioavailable and mobile, and are therefore dangerous.

Palladium is considered the most toxic due to its high solubility and mobility, followed by platinum. The mechanism of action and fate of PGM in the environment is still not completely clear. Relatively few studies are devoted to the toxicity and at the same time bioaccumulation of PGM. These studies are mostly not done under standardized conditions, which makes it impossible to compare the results. The aim of the work was to perform both ecotoxicological tests and monitor the bioaccumulation of the most toxic and risky elements from the group of platinum metals, namely palladium (Pd) and platinum (Pt), on selected organisms (green algae *Desmodesmus subspicatus*, aquatic crustacean *Daphnia magna*, white mustard *Sinapis alba*). These organisms are representatives of different trophic levels and are model organisms in ecotoxicological tests. Ecotoxicological tests were carried out under standard conditions according to the relevant ISO standards. Inhibition of growth rate after 72 hours was evaluated for green algae, immobilization after 48 hours for daphnia, and inhibition of root growth after 72 hours for mustard. After performing the ecotoxicological tests, all tested organisms were washed with ultrapure water, dried and digested in a microwave digestion instrument and analyzed with ICP-MS/MS device. The results show that the least sensitive organism to Pt and Pd is mustard, for which approximately the same toxicity of Pt and Pd was found and the EC50 values were an order of magnitude higher than for algae and daphnia. Although the toxicity of both Pt and Pd to mustard was approximately comparable, mustard was found to accumulate significantly more Pt compared to Pd. Pd was more toxic than Pt to green algae, with more Pt being observed to accumulate in algal cells. On the other hand, Pt was more toxic to daphnia with a lower degree of bioaccumulation in organisms than Pd. The results of bioaccumulation studies show the different ability of individual organisms to accumulate platinum metals. The information obtained can be used for risk assessment and in the bioremediation of wastewater.

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3.04.P-Th103 Assessment of Acute Toxicity in *Daphnia magna* Exposed to Binary and Ternary Metallic Elements Mixtures of As, Cu, Zn, and Se: Experimental Investigations and Modeling Strategies

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This study investigates the impact of contaminant mixtures from mining and metallurgical effluents on *Daphnia magna*. Acute toxicity tests were conducted on metals (Cu and Zn) and metalloids (As and Se), individually, in pairs, and in ternary combinations. The results of binary mixtures revealed varying levels of toxicity: lower than expected (As-Se and As-Zn), consistent with predictions (As-Se and Zn-Se), or higher than anticipated (Cu-Zn and Cu-Se). When combined into ternary mixtures, the interactions between elements introduced additional complexity, influencing *D. magna* toxicity in different ways. The As-Cu-Zn and Cu-Zn-Se mixtures exhibited moderate to high toxicity, primarily driven by the synergistic effects of Cu-Zn and Cu-Se, while the As-Zn-Se and As-Cu-Se mixtures showed lower to moderate toxicity, potentially due to an attenuating effect of As in certain interactions. These findings underscore the importance of evaluating complex metal interactions to better understand their impact on aquatic ecosystems and to guide environmental management policies.

3.04.P-Th104 Ecotoxicity of Field-Realistic Metal-Organic Mixtures to the Freshwater Algae *Raphidocelis subcapitata*

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Current environmental risk assessment frameworks predominantly rely on a substance-by-substance approach, even though chemical contamination in the environment typically occurs as complex often unknown mixtures. Regulatory agencies are increasingly recognizing the need to assess the risks associated with chemical mixtures and to systematically integrate combined exposure and effects into chemical risk assessments.

This study investigates the aquatic toxicity of real-world metal-organic mixtures. We selected four sampling sites in rivers and streams in Belgium, chosen for their proximity to wastewater discharge and industrial effluent sources to ensure the presence of both metal and organic contaminants. At each site,

samples for metal analysis (filtered at 0.45 µm) were collected and Large Volume Solid Phase Extraction was used to isolate and concentrate organic micropollutant mixtures.

We conducted 72-hour growth inhibition tests for each site with the freshwater algae *Raphidocelis subcapitata* (OECD 201). We first performed range-finding tests to estimate the toxicity of the reconstituted metal and organic mixtures by testing them at different relative enrichment factors (REF). The REF is the metric used for expressing the concentration or dilution of a mixture of contaminants relative to their environmental level, while maintaining their relative proportions always constant. A REF of 1 means that the mixture is tested at the same concentration as in the sampled body, while a REF of 10 increases all components of the mixture to 10 times their environmental concentrations. We then conducted binary mixture toxicity tests to assess the interactions between the metal and organic mixture groups.

We used a specific experimental design to test two key hypotheses: (1) that independent action provides a more accurate predictive model than concentration addition for toxicity of environmentally relevant metal-organic mixtures, and (2) that complex organic mixtures present at no-effect concentrations do not influence metal toxicity, and vice versa. If the second hypothesis holds, it would suggest that metals and organic chemicals could be assessed independently in regulatory frameworks.

We aim that this study would provide relevant experimental data to improve our understanding of the joint toxicity of environmentally and regulatory relevant metal-organic mixtures, allowing a scientifically more robust risk assessment of chemical mixtures.

3.04.P-Th105 The Impact of Metal Mixtures on Local Biodiversity – An Analysis of Flemish Freshwater Monitoring Data

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Biodiversity loss is influenced by a complex interplay of environmental factors, yet the impact of anthropogenic chemicals in general and metals in particular on local biodiversity and how it relates to other environmental factors remains poorly understood. Additional complications are that metals in the environment occur in mixtures and their bioavailability essential to determine their toxicity varies depending on water chemistry conditions. To better understand the impact of metals on local biodiversity, we analyzed freshwater monitoring data from Flanders, including water chemistry, metal concentrations and biota (macroinvertebrates and phytobenthos). The objective of the study is to determine whether metal pressure can be used to explain local biodiversity trends and which biodiversity indices are most sensitive to metal pressure.

To quantify the expected impact of metals, metal concentrations were corrected for bioavailability and used to calculate metal mixture pressure indices based on single metal toxicity data. To quantify biodiversity, macroinvertebrate and phytobenthos counts were used to calculate a range of biodiversity indices (e.g., Shannon-Wiener index, species richness). Additionally, trait-based indices, based on species traits such as life cycle type or feeding mechanism, were calculated. Statistical methods were used to determine whether any biodiversity indices were significantly explained by metal pressure and if there were safe mixture pressure thresholds below which no effects on biodiversity are expected. Statistical trends were further explored by subsetting the data e.g., based on water body type or location.

The findings of this study enhance the understanding of metals as potential drivers of biodiversity loss and will inform future risk assessments.

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3.04.P-Th106 MEED: Progress with the Multiyear Metals Environmental Exposure Data Collection Program to Anticipate Challenges of the EU Zero Pollution Ambition Policy and the Chemicals Strategy for Sustainability

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The Chemicals Strategy for Sustainability (CSS) is a key pillar of the EU Zero Pollution Ambition (ZPA), a main building block of the Green Deal. This policy aims for achieving good quality status of the environment by achieving exposure levels of chemicals that are no longer harmful. This challenges chemicals regulations given focused on substance specific assessments and limits. The combined effects of chemicals in the environment and assuring they do not affect biodiversity, needs therefore to be included in regulatory schemes. The EU, proposes the introduction of a Mixture Allocation Factor (MAF)

to demonstrate safe use and lack of impact on ecosystems from a cocktail of chemicals exposures. This is a real scientific challenge for the EU industry, especially for metals given their increasing volume and use in Green Deal applications like electric vehicle batteries, solar cells, windmills and electronics. This challenge drove the metals sector to design a comprehensive Metals environmental exposure gathering programme (MEED), complemented by the development of scientific concepts and selective test work to assess combined exposure. All to promote we can assess progress toward the ZPA and the EU biodiversity objectives. MEED runs for 4 years up to 2025, covering 6 interlinked projects and enters its final phase. The identification of metals that contribute the most to the combined risks in EU soil and aquatic environments was a first milestone followed by an extensive investigation of today's regional background levels for metals, and an update on the STP emission contribution as a key source of consumers input. A review and reappraisal of existing knowledge on metals mixtures & metals-organics mixtures interactions allowed to define gaps and the design of a gap filling research on mixture toxicity as well as a tiered assessment system for local and regional biodiversity impact assessment. The main outcomes of this R&D are now becoming available allowing to design a science-based concept to improve the assessment of combined effects for regulatory schemes like REACH or the EU-Water Framework Directive. This poster presents the progress made and today's conclusions of the program while detailed assessment of key new milestones are presented in complementary posters. MEED is delivering a huge amount of data and relevant concepts to be used in REACH registration updates and key to assess progress with the ZPA policy of the EU in a scientific way.

3.04.P-Th107 Updating the Bioavailability-Based Environmental Quality Standard Derivation Approach for Compliance Assessment of Zn in Freshwaters

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Many countries list Zn as a River Basin Specific Pollutant under the EU Water Framework Directive (WFD). Given the dependency of the toxicity of Zn on local water body conditions, it is essential that the derivation of Environmental Quality Standards (EQS) for Zn are bioavailability-based. In the present study, the existing bioavailability-based EQS derivation approach, which has been widely used and accepted for more than a decade; was updated by I) revising the existing ecotoxicity dataset, and updating it with recent high-quality ecotoxicity data for freshwater organisms, including species that were previously not considered and II) optimising the bioavailability models in terms of model structure and speciation software. The updated EQS derivation approach was then used to derive a bioavailable EQS for Europe, which can serve as a first-tier screening level in compliance assessments.

The updated chronic Zn freshwater ecotoxicity dataset combines high-quality data retained from the existing Zn freshwater toxicity datasets with high-quality data identified in a recent literature screening. The updated database now contains data covering 40 species and covers the minimum data requirements for species sensitivity distribution-extrapolation set out in the relevant guidance of the WFD. The existing chronic Zn bioavailability models were revised by optimizing the model structure and using the most recent version of WHAM as the underlying speciation software. Within the validation of the updated models with independent data for nine aquatic species: EC10 were generally predicted within 2-fold error for model-species and within 3-fold error for non-model species with few exceptions. Hence, the updated bioavailability models can be reliably used to accurately predict Zn toxicity across different species. The updated ecotoxicity dataset and the updated chronic bioavailability models were combined with species sensitivity distribution techniques resulting in the updated EQS derivation approach for Zn, which allows the derivation of a site-specific environmental threshold for Zn. The updated EQS derivation methodology has been used as a basis to derive a robust bioavailable EQS for Zn in European freshwaters. The bioavailable EQS in combination with the updated bioavailability normalisation approach will improve the environmental relevance of compliance assessment for Zn under the WFD.

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3.04.P-Th108 Derivation of a Bioavailable Environmental Quality Standard for Copper

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The toxicity of Cu to aquatic organisms depends on local water body conditions, with dissolved organic carbon, pH, hardness and sodium as main toxicity modifying factors. Cu is a river basin specific pollutant

under the Water Framework Directive. For metals with validated bioavailability models, compliance is assessed using a tiered approach based on a bioavailable environmental quality standard (EQSbioavailable). The EQSbioavailable is set at the environmental threshold that protects 95% of the waters in the European country with the highest bioavailability conditions (or highest Cu sensitivity). The current EQSbioavailable (1.0 µg dissolved Cu/L) is based on a bioavailability-based derivation approach, which exists for more than a decade. Here, we present an updated bioavailability-based normalisation approach for Cu that incorporates recent updates in ecotoxicity data and bioavailability models, and we assess whether the current EQSbioavailable is still valid.

The proposed chronic Cu freshwater ecotoxicity dataset combines high-quality data retained from the existing Cu freshwater toxicity datasets with high-quality data identified in a recent literature screening. The updated database contains 49 species and is among the most data-rich ecotoxicity databases for metals. This dataset is combined with recent chronic Cu bioavailability models and species sensitivity distributions techniques in a dedicated, easy to use bioavailability normalisation tool, CuBioM. CuBioM calculates site-specific environmental thresholds for European surface waters, which were used as basis to derive a EQSbioavailable for Cu following the relevant guidance. Preliminary calculations suggest that the updated ecotoxicity dataset and bioavailability models confirm the validity of the current EQSbioavailable of 1.0 µg dissolved Cu/L. Next, the updated bioavailability models and ecotoxicity dataset will be included in bio-met. In practice, compliance of water bodies with the EQSbioavailable for copper is best assessed using either CuBioM or bio-met. This improves the environmental relevance of the assessment by incorporating bioavailability.

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3.04.P-Th109 Compliance Assessment of Cu under the Water Framework Directive

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Cu is a river basin specific pollutant (RBSP) under the European Union (EU) Water Framework Directive (WFD). Cu has been reported by the European Environmental Agency as one of the RBSP that most frequently fails compliance against the environmental quality standards (EQS). The toxicity of Cu to aquatic organisms is dependent on local water body conditions (such as Dissolved Organic Carbon, pH, hardness and sodium), and therefore it is essential that compliance assessment of Cu is bioavailability-based. However, it is not clear whether, and to what extent bioavailability is incorporated in current compliance assessments. Therefore, the risks of Cu may be over- or underestimated.

Here, we present an EU-wide compliance assessment of Cu in freshwaters, following the tiered approach set out in the relevant WFD guidance, and based on the most recent ecotoxicity database and optimisations in Cu bioavailability models. These have been integrated in CuBioM, an easy-to-use bioavailability normalisation tool, that allows to calculate site-specific environmental thresholds for Cu, expressed as the 5% hazardous concentration. We perform a Tier 1 compliance assessment against the EQSbioavailable, and a Tier 2 bioavailability corrections based on local water chemistry conditions. While a considerable proportion of waters fail the Tier 1 assessment, the study highlights how incorporating bioavailability in Tier 2 if the compliance assessment increases the environmental relevance of compliance assessments. This suggest that a Tier 1 assessment does not really help to screen out sites for copper, and it may be more efficient for regulators to directly assess compliance against a site-specific EQS under Tier 2. In addition, the extent of Cu exceedances when incorporating bioavailability will be discussed. The bioavailability correction using CuBioM serves as a robust approach which can aid regulators to improve environmental relevance when performing local compliance assessments for Cu under the WFD. The multi-metal tool bio-met, which was developed to assess compliance of metals under the WFD, will next be updated to reflect the updated approach for copper.

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3.04.P-Th110 Nickel Water Quality Guideline Updates in Canada

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The Canadian Council of Ministers of the Environment and British Columbia Ministry of Water, Land, and Resource Stewardship have developed new sets of nickel water quality guidelines for the protection of aquatic life in freshwater and marine environments over short-term and long-term exposures. These

guidelines replace an old hardness-based guideline for long-term nickel exposure in freshwater that has been the only Canadian nickel guideline since 1987. The guideline updates allowed for the consideration of bioavailability effects and new toxicity data produced since the old guideline was created. Toxicity databases were updated to form species sensitivity distributions (SSDs) for each of the four guidelines, including 29 and 34 freshwater organisms for short-term and long-term SSDs, respectively, and 31 marine organisms in each the short-term and long-term SSDs. The normalized SSDs are fit with three probability distributions (lognormal, log-logistic, and gamma), and the model average is used to calculate a best-fit 5th-percentile value as the basis for the guidelines. The new freshwater guidelines use biotic ligand model (BLM) normalization, with software providing the official guideline values as well as a conservative estimate based on fewer chemistry inputs, and lookup tables providing easier access to estimated guideline values. Three organisms in the freshwater SSDs were seen to have substantially lower nickel effect concentrations at high pH, likely due to added toxicity from bicarbonate, which lead to the decision to use a separate set of model parameters for those organisms. The freshwater guideline values generally increase with higher dissolved organic carbon and hardness, and decrease slightly with higher pH, although the relationships are very non-linear. This is because the BLM is a chemical speciation model, and two different parameter sets are used within the SSDs. The marine guidelines are based on a simple log-linear equation with salinity as the only toxicity modifying factor. Therefore, the marine guideline values increase exponentially with linearly increasing salinity and can be calculated using a simple equation. These updates to Canadian guidelines come at a time when both the United States and Europe are working to update their own nickel water quality standards, and Canada can be used as an example of how another jurisdiction is using the same data and scientific advancements to address the same environmental problem.

3.04.P-Th111 The Impact of Climate Change on the Flux and Fate of Metals in Freshwaters: Implications for Metal Bioavailability Across Different Scales

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Climate change and chemical pollution are two of the gravest environmental concerns, and it is increasingly recognised that climate change and climate variability will alter the environmental distribution and toxicity of chemical pollutants, including in the aquatic environment. Trace metals are an established contaminant group where decades of research have been able to determine causal links between environmental concentrations, water chemistry and bioavailability, and accumulation and toxic effects. In freshwaters, these relationships have been parameterised into models for some metals, most notably Biotic Ligand Models (BLMs), that have utility to normalise toxicity values to site-specific water chemistry. The assumptions that such models are predicated upon need to be tested in increasingly climate variability. Here, we assert that to fully comprehend the impact of climate change on metal bioavailability in freshwaters, three distinct scales need to be understood: (1) the global scale of metal biogeochemical cycling which will alter metal inputs into freshwaters as climate change affects soil properties, hydrological connectivity, and metal mobility; (2) the environmental scale of fluctuating water chemistry parameters, such as dissolved organic carbon inputs, that will change free ion availability; and (3) the organismal scale at which climate change will push organisms outside of their tolerance window, inducing physiological modifications that alter the bioaccumulation and toxic effects of metal exposure. By understanding the impact of climate change on metals at each of these scales, and the connection between them, it is possible to further understand the future needs for metals regulation.

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3.04.P-Th112 Impact of Diatom-Produced Extracellular Polymeric Substances on Silver Nanoparticle Behavior in Freshwater

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Silver nanoparticles (nAg) are widely used across multiple fields, and they can be released into freshwater. Once into aquatic environment, their fate can be shaped by various environmental factors. Among these, extracellular polymeric substances (EPS) produced by aquatic micro-organisms represent a significant but underexamined element in the fate of nAg. This study explores how EPS derived from the diatom *Cyclotella meneghiniana* interact with 20 nm citrate-coated nAg, influencing their colloidal stability, surface properties, and dissolution in freshwater condition. The research employed a combination of

techniques to analyze nAg behavior when exposed to EPS. Experiments were conducted over both short (0-2 hours) and long (up to 72 hours) term in a simulated synthetic freshwater medium. Additionally, different EPS concentrations were tested to evaluate potential concentration-dependent effects on nanoparticle behavior. EPS were rich in polysaccharides and proteins, which play a key role on nAg interaction. The EPS adsorbed onto the nAg surfaces, creating a stabilizing layer that enhanced colloidal stability by inducing steric repulsion. This eco-corona formation significantly reduced homo-aggregation processes which occurred in the absence of EPS due to effect of mono and divalent ions. Interestingly, EPS also promoted slight heteroaggregation processes, with higher EPS concentrations correlating with the formation of larger aggregates, probably through a bridging effect of adsorbed polysaccharides. EPS were shown to influence the dissolution of nAg by eco-corona. This eco-corona hindered the release of silver ions, which are the primary contributors to the toxicity of nAg. Transmission Electron Microscopy imaging confirmed the formation of this eco-corona, using negative staining with uranyl acetate to highlight the interaction between EPS and nAg. Both polysaccharides and proteins were involved in the hard corona formation, with proteins more strongly implicated in ecocorona formation as demonstrated by fluorescence quenching, which indicated hydrophobic interactions between EPS and nAg. In conclusion, EPS released by *C. meneghiniana* play a crucial role in determining the environmental fate of silver nanoparticles. By enhancing colloidal stability, mitigating homoaggregation, and reducing dissolution rates through ecocorona formation, EPS was showed to be able to influence the behavior and potential ecological impact of nAg in freshwater environments.

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3.04.P-Th113 In Vitro Biotransformation of Silver Nanoparticles by Freshwater Phytoplankton
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Silver nanoparticles (AgNPs) are widely used due to their strong biocidal properties, leading to their inevitable release into aquatic environments. Once introduced, AgNPs can persist in the water column for extended periods, with environmental factors influencing their stability and interactions with aquatic organisms. While considerable research has focused on the toxicological effects of AgNPs on aquatic biota, the mechanisms by which phytoplankton handle and transform AgNPs remain poorly understood. This research explores the biotransformation of AgNPs by two phytoplankton species: *Cyclotella meneghiniana*, a diatom, and *Chlamydomonas reinhardtii*, a green alga. Biotransformation is expected to occur once AgNPs cross the biological membranes. This study focuses on the dynamics of biotransformation, including surface properties induced by bio-corona formation, and nanoparticle dissolution, through in vitro experiments up to 24 hours. Bio-corona formation was investigated using asymmetric flow field-flow fractionation coupled with ICP-MS (AF4-FluoD-ICP-MS) and bio-transformed AgNPs were analyzed by time-of-flight secondary ion mass spectrometry (TOF-SIMS). The results revealed species-specific bio-corona formation, with larger hydrodynamic sizes and altered zeta potentials of bio-transformed AgNPs. The formation of the bio-corona was confirmed by AF4 coupled with ICP-MS, where a clear colocalization of proteins with nanoparticles was observed. Finally, TOF-SIMS analysis detected protein fragments as well as Ag_x(CN)_y species, confirming the presence of surface-bound proteins and supporting the evidence of bio-corona formation.

Furthermore, significant increases in the dissolution of AgNP was observed in both organisms extracts following the bio-corona formation, suggesting that the adsorbed biomolecules may facilitate the oxidative dissolution of AgNPs. This biomodification could enhance AgNP toxicity, as dissolved silver ions (Ag⁺) are known to be the primary drivers of AgNP toxicity. On the other hand, this biomodification might reduce the transfer of AgNPs through the aquatic food chain.

Overall, this study underscores the critical role of freshwater phytoplankton in the handling and biotransformation of AgNPs, providing valuable insights into their fate in the aquatic environment and their potential impact on the aquatic ecosystem.

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3.04.P-Th114 Tracing Microbiome Proteins in Ecocoronas: A Case Study for Titanium Dioxide Nanoparticles

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In any internal or external environment, metal nanoparticles sequester biomolecules from their immediate surroundings through chemi- and physisorption interactions. Collectively, these biomolecules form a transient face on the particle surface, termed the biocorona or ecocorona, dynamically shaping the interactions between nanoparticles and their surroundings. Due to the transient nature of these interactions, the origin of many ecocorona proteins is unknown. We hypothesized that microbiome proteins form an important source of biomolecules for the formation of ecocoronas, given the abundance of host-associated microbes, and their activity in many host-associated processes. We set out to test this experimentally for titanium dioxide nanoparticles (nTiO₂), by incubating these particles in test medium that was pre-incubated with daphnid neonates (*Daphnia magna*) and zebrafish larvae (*Danio rerio*). I will present how we combined metagenomic and proteomic data to trace up to 192 daphnid proteins, 650 zebrafish proteins, and 1405 and 822 proteins of their respective microbiomes in the acquired ecocoronas. Our study revealed that microbiome proteins occupy a large proportion of the available binding spaces on the particle surface, and exhibit distinct physico-chemical properties from host proteins. Moreover, our study showed that the binding targets that have been reported for these proteins, differ between *D. magna* and *D. rerio* ecocoronas. The results of this study exemplify how microbiome proteins can alter the surface properties of metal nanoparticles, and reveal potential implications on particle stability, adsorption and binding interactions.

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3.05 Developments, Challenges and Solutions in Chemical (Bio)Degradation and Persistence Assessment

3.05.T-01 Regulatory Challenges and Needs in Degradation Testing and Persistence Assessment of Difficult to Test Substances

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Persistence assessment is a crucial element within the context of hazard and risk assessments under many regulatory frameworks. Data from studies that simulate various environmental compartments such as surface and marine water as well as sediments and soils are preferred. In addition, screening level tests (ready and inherent biodegradability tests) can be also employed as part of the (bio)degradation assessment strategy. Assessing persistence of difficult to test substances can be challenging due to their properties (e.g., volatility, low solubility, ionisation, complex composition etc.). Testing guidelines have limited applicability for the biodegradation testing of such substances and this hampers regulatory decision-making. Thus, there is a pressing need to provide more guidance on how to improve the quality and relevance of the data produced via the standard test methods. So far, several attempts have been made to address the issues encountered during the degradation testing for such substances (Birch, H., et al., 2023; Cefic, 2022; ECHA, 2023a; ECHA, 2023b; UBA, 2023). Further modifications to the standard test methods are needed along with the development of new approaches and methodologies. Such approaches, amongst others, could focus on specific routes for introducing the test chemical into the test system, new reference substances, benchmarking, inoculum/biomass characterisation, developing robust analytical methods, the use on non-specific testing methodologies, optimisation of test system, e.g., trapping of volatiles, development of predictive models for addressing degradation and advancing the methods for the degradation assessment of UVCB substances. From a regulatory perspective, it is critical that these modifications should focus on improving the repeatability and applicability of the test system to meet the specific objectives of the assessment. Enhanced guidance and improved testing guidelines would benefit authorities and also drive innovation toward the creation of safer and sustainable chemical products. This presentation will provide an overview of the recommendations on the degradation testing and persistence assessment of difficult to test substances included in ECHA Guidance on IR&CSA (Chapters R7b and

R.11). It will outline still existing regulatory challenges in the persistence assessment of difficult to test substances, drawing on insights from past evaluations and recent scientific literature.

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3.05.T-02 Building Knowledge from Available Degradation Simulation Studies to Improve Use in Regulatory Persistence Assessments

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Approximately 400 higher tier persistence studies are reported in REACH dossiers (eChemPortal.org). The number of regulatory requests for such information are increasing. For example, in the period 2020-2023, over 300 simulation tests were requested through the REACH dossier evaluation (DEv) process. Higher tier persistence studies, namely the OECD test guidelines (TGs) 307, 308, and 309, are technically challenging for many chemicals, costly and lengthy to perform. It is imperative that they provide usable robust and reliable data. Substances that have challenging physical chemical properties add to the complexity of performing and interpreting generated data, which must be usable for both environmental exposure and persistence assessments across different regulations. In this context, a database has been developed to capture metadata from surface water degradation simulation studies (OECD TG 309). A total of 168 substances having reliable OECD TG 309 degradation simulation studies were identified. All relevant metadata for each study was collated in a database that was designed in line with the Persistence Assessment Tool. The range of reported degradation half-lives encompassed very low values (potentially due to abiotic processes such as hydrolysis) to very high values (potentially default half-lives where little to no degradation is observed during the study). Various statistical analyses are being conducted on this dataset, and half-lives are being compared with different predictor variables such as physico-chemical properties (e.g., water solubility, volatility), structural characteristics (Ecosar classes, Biowin fragments) or test system parameters (e.g., temperature, test volume). The database allows for detailed statistical analysis to provide an understanding of test system parameters or substance physicochemical properties that significantly influence study outcomes; and will aid in the interpretation of data. Further to this, it should identify whether current practices are relevant and accurate or if they need to be updated.

3.05.T-03 Does Benchmarking Increase the Accuracy of Predicting Biodegradation Across Aquatic Ecosystems?

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Biodegradation is often the largest source of uncertainty in assessing chemical exposure. Previous studies have reported significant spatial and temporal variability in biodegradation rates, yet no environmental factors have been identified to mechanistically explain this variability. This study compiled a comprehensive dataset of 2265 biodegradation rate constants for 97 diverse chemicals from modified OECD 309 tests in 38 European and Australian aquatic ecosystems. We evaluated three approaches for reducing/describing spatiotemporal variability in rate constants: total microbial biomass normalization (TBN); universal benchmarking (UBM), i.e., normalizing all chemicals to a single benchmark chemical; and group-specific benchmarking (GBM), i.e., normalizing chemicals within each chemical group to a single benchmark chemical selected from that group. Neither TBN nor UBM reduced the variability, while GBM reduced the variability for 87% of compounds when grouping was based on clustering of spatiotemporal patterns of rate constants. However, GBMs based on chemical features (MACCS molecular fingerprints or predicted initial biotransformation rules) had negligible effects in reducing the variability. Three chemical groups that have high structural similarity showed considerable reduction in variability using both data-driven and chemical feature-based GBMs. We conclude that chemical grouping

has the potential to predict biodegradation rates across ecosystems. However, for group-specific benchmarking to become a viable predictive tool, a deeper understanding of the key chemical features associated with biodegradability is essential.

3.05.T-04 Opportunities and Limitations of Data-Driven Models to Predict Environmental Persistence

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Innovative methods are essential for accurate and efficient chemical exposure assessment, especially given challenges like testing numerous substances and accounting for transformation products. Current structure-activity models often lack the accuracy needed. This study explores machine learning (ML) to address these limitations, focusing on predicting two environmental persistence drivers: biotransformation half-lives (DT50) in soil and breakthrough rates in wastewater treatment plants (WWTPs). These models fill gaps in traditional testing, supporting safe-and-sustainable-by-design (SSbD) approaches for rapid chemical evaluation.

DT50 data from enviPath and breakthrough data from 44 WWTPs across three countries were processed using PEPPER, a computational workflow developed by our group and presented for the first time in this work. Six molecular representations, including PaDEL, Mordred, and RDKit-based fingerprints, were combined with 12 ML algorithms ranging from linear models to advanced methods like Random Forest and Gaussian Process Regressor.

Results showed that Gaussian Process Regressor, Support Vector Regressor, and Random Forest performed best, though accuracy remains limited by experimental variability. RMSE for breakthrough predictions was ~0.7 log units, while DT50 predictions had RMSE of ~0.8 log units. Compared to existing tools like STPWIN of the EPI Suite, our models had significantly lower RMSE, demonstrating superior capability in capturing complex processes like micropollutant removal in WWTPs.

The study also highlights the importance of confidence metrics for prediction reliability. Unlike traditional definitions of the applicability domain, we propose confidence metrics based on prediction variability. This provides more nuanced, accurate, and trustworthy insights which we believe are essential for future users. We validated this metrics and demonstrated that significantly lower errors are achieved for the predictions with the highest confidence.

This work showcases the potential of machine learning to enhance environmental persistence assessments, addressing current tool limitations and providing novel tools for reliable chemical persistence and risk evaluation.

3.05.P-We209 Polymer Biodegradability and the Link Between Abiotic and Biotic Degradation

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Polymer persistence is a growing environmental concern, and current biodegradability tests are often designed for small molecules and may not adequately capture the complexities of polymer degradation. This study evaluates the impact of hydrothermal pre-treatment on polyester biodegradability using a novel assessment method combining pre-treatment and advanced physicochemical and chemical analyses in parallel to biodegradability testing, enabling the identification and monitoring of specific degradation products.

Polyhydroxybutyrate (PHB) and polylactic acid (PLA) served as model biodegradable and non-biodegradable polymers, respectively. Hydrothermal ageing (130°C) was performed for varying durations (1-120 hours). Following hydrothermal ageing, water-soluble degradation products were analyzed directly by Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) to characterize the initial breakdown products. Subsequently, OECD 301F biodegradability tests were conducted on pristine and aged PHB and PLA particles, extracted water-soluble oligomers, and the corresponding monomers. To analyze the degradation products formed during these biodegradability tests, Solid Phase Extraction (SPE) cartridges were employed for efficient extraction from the complex biodegradability medium before LC-HRMS analysis.

The analysis confirmed the presence of PHB oligomers of up to 5 repetition units in hydrothermally aged samples. These same oligomers were identified at a strategically chosen 5th day of the biodegradation test. Hydrothermally aged PHB showed enhanced biodegradability (shorter lag phase, faster 60% threshold achievement), while PLA remained non-biodegradable. The contrasting responses of PHB and PLA

suggest a possible correlation between the extent of hydrothermal degradation and biodegradability. This finding indicates a potential rapid screening test methodology for polyesters, which will be further investigated with additional biodegradable polymers upon solubilization/improved bioavailability such as polycaprolactone (PCL). This improved assessment approach advances our understanding of polymer environmental fate.

3.05.P-We210 Identifying Bound Compounds in Non-Extractable Residues of Pesticides in Soil by 4-Pool Kinetic Analysis

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Non-extractable residues (NERs) of pesticide in soil are one of the major uncertainties in pesticide risk assessment and persistence evaluation for PBT and PMT classification, because of their unknown chemical identifies. This study explores the feasibility of identifying bound compounds in non-extractable residues (NERs) of pesticides in soil by 4-pool kinetic analysis. The 4-pools refer to parent compound, metabolites, NERs, and CO₂ in 14C-labeled pesticide soil degradation studies. We discovered the following two characteristic 4-pool kinetic behaviors of formation of NERs: (1) if parent compound is bound as NERs, the metabolites (m(t) in % applied radioactivity (AR)) kinetically drive the evolution of CO₂ only; and (2) if a metabolite (x) in a sequential degradation pathway is bound as NERs, m(t) is split into m1(t) and m2(t) at the metabolite (x) that is bound as NERs, which kinetically drive the formation of NERs and evolution of CO₂ respectively. We developed two (i.e., Parent->NER and metabolite->NER) 4-pool models to capture the kinetic behaviors respectively. By fitting the models to a set of 4-pool data, we not only determine whether NERs form from parent compound or metabolites but also identify the bound metabolite (x) by resolving the metabolites into M1(t) and M2(t) (i.e., the variables used to simulate m1(t) and m2(t)) and then matching m1(t) (i.e., the sum of bound metabolite (x) and its metabolite precursors) with M1(t). By applying the models to 14C-labeled parent compound-dosed studies and then sequentially to metabolite-dosed studies for 7 pesticides with ~70% AR NERs, we identified the bound compounds, which have the moieties known to be responsible for NERs, are bound as NERs instantaneously when dosed into soil, and account for pH dependency of NER formation. This study demonstrated that we could identify the bound chemical species in NERs of pesticides, assess the risk/hazards of NERs at a molecular level, and thereby eliminate the uncertainties in pesticide risk and persistence assessments.

3.05.P-We219 Probabilities to Prioritize Potential Persistent Pollutants

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Chemical hazard and risk assessments often use physical-chemical properties to categorize and identify chemicals of concern. In Europe, recent legislation regarding the classification, labelling and packaging (CLP) of consumer products includes new chemical hazard categories, including persistent, mobile, and toxic (PMT) and very persistent and very mobile (vPvM). Persistency is currently assessed using ready biodegradability data and half-lives of the substance in different systems. This is additionally challenging because of the limited availability of data and information in the environment. We combine available datasets available in literature, data from OECD's eChemPortal and QSAR Toolbox and predict ready biodegradability and degradation half-lives using QSAR models such as OPERA and EPI Suite. We combine data, including availability of uncertainty data and errors, and the relative reliability of the source of data using Bayesian analysis methodologies.

In this work we assess the potential persistence of substances from the ZeroPM Global Chemical Inventory, which includes approximately 119,000 SMILES that represent 93,000 substances. We predict the probability that a substance is not persistent, persistent, or very persistent based on the available data. This data can be used as part of a weight of evidence approach when the probability of any one category is very high or to identify substances where further experimental testing and reporting is required because the probability of a substance being not persistent is near 50% and thus the results are inconclusive. We also explore the entire dataset and the cumulative probability scores, to understand how many of the chemicals registered on the global market may be considered potentially persistent or not persistent.

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3.05.P Developments, Challenges and Solutions in Chemical (Bio)Degradation and Persistence Assessment

3.05.P-We195 Paving the Path for A Revision of the OECD Tests on Ready Biodegradability by A Round Robin Test

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Standardised test systems offer constant test conditions as well as the possibility of generating comparable and reproducible test results. The tests of the OECD 301 series and the OECD 310 test are standardised and equally valid as screening tests on ready biodegradability. However, though standardised and the procedure used being similar, they differ from each other in important aspects. On the one hand the reason is historical: the majority were developed in the 1980s in various OECD member states, mostly independently of each other. On the other hand, their test conditions represent the technical possibilities of the time. Even the revisions of the test guidelines in 1992 did not fundamentally change the co-existence of the different variants.

All screening tests on ready biodegradability have a relatively simple test design and are inexpensive compared to simulation tests. They thus represent the first information layer on biodegradation which is important because further assessment procedure depends on the data generated here. However, observed differing and sometimes contradicting results between the different test systems is criticised. With the further development of analytical technology since 1992, but also through the preferential use of individual test systems, it should be possible to completely dispense with test systems that are now rarely used and to better standardise and adapt the framework conditions of the remaining test procedures to each other. This would also make sense as it would improve the comparability of test results between different substances.

The aim of this project is to generate data for a subsequent revision of the OECD tests for ready biodegradability. A proposal for a consolidated test design is developed and put to the test. To this end, the contractor is carrying out an international round robin test. At least ten GLP-qualified laboratories should participate and test three substances. In the course of the project, it is planned to present and discuss the results in an international workshop. The project started in September 2024.

3.05.P-We196 Consolidation of OECD Tests for Ready Biodegradability on the Example of OECD 310

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Standardized test systems ensure constant test conditions and facilitate generating comparable and reproducible test results. The OECD 301 A-F/ OECD 310 series for testing ready biodegradability are considered equally valid. The tests are routinely used both for the investigation of ready biodegradability and for the identification of non-persistent substances within the PBT-assessment. However, the guidelines have not been revised for quite some time. The last substantial revision was in 1992 for OECD 301 A-F and in 2006 for OECD 310. Revising, updating and consolidation of the current guidelines is therefore a recurring topic of discussion.

Among the different ready type tests the OECD 310 represents a broad range of boundary testing conditions: The allowed test concentration range is from 2-40 mg/L TOC (20 mg/L TOC preferred), The allowed inoculum sources are activated sludge (4-30 mg d.s./L 4 mg/L d.s. preferred), secondary effluent (up to 100 mL/L), surface water and soil. Among the points which require further clarification are:

- The inoculum concentration should result in 10^2 to 10^5 CFU/mL in the final mixture (colony-forming units), but there is no indication of the method for determining the CFU. Due to the inhomogeneous and flocculent structure of the activated sludge, CFU determination is critical.
- One validity criterium refers to the inorganic carbon allowed in the inoculum blank control which must be < 3 mg C/L at the end of the test. This criterium is only achievable when the standard concentration of 4 mg/L d.s. activated sludge is applied. The validity criterium should reflect both the test concentration and the inoculum concentration.
- The incubation temperature of the OECD 310 is $20^\circ\text{C} \pm 1^\circ\text{C}$, which is different from the incubation temperature of the OECD 301 tests ($22^\circ\text{C} \pm 2^\circ\text{C}$)

Own experiences with different testing conditions reflecting the liquid/headspace ratio and the substrate/inoculum ratio are being presented. The OECD 310 provides an appropriate and variable test system allowing biodegradability testing under both ready and inherent type conditions. The version under inherent test conditions resembles the inherent test proposed by CONCAWE in 1999 (draft OECD 302D) for lubricants, but applies standard inoculum without the option to use preadapted inoculum sources which has been pre-exposed to the test substance.

3.05.P-We197 Ready Biodegradability Studies: OECD 301 or 310?

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Ready biodegradability experiments are laboratory tests designed to evaluate the potential of a chemical to

undergo rapid and complete degradation by microorganisms under aerobic conditions. These tests simulate natural aquatic environments, measuring the conversion of organic carbon into CO₂, water, and biomass. They provide an early indicator of a chemical's environmental persistence and its likelihood of bioaccumulating or causing long-term harm to ecosystems.

OECD 301F and 310 are two of the internationally accepted protocols for conducting ready biodegradability experiments. While the OECD 301F study design measures the amount of oxygen consumed by microorganisms during the degradation of the test substance to calculate degradation, the OECD 310 study measures the amount of CO₂ produced.

Under the European Union's REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals) regulation, enhancements to standard biodegradability tests like OECD 301F and OECD 310 are sometimes implemented to address specific regulatory or scientific needs. These enhancements are designed to provide more environmentally realistic data or better assess challenging substances. Key modifications include larger test vessels and extended test durations.

The ready biodegradability of a compound of interest has been determined using both OECD 301F and 310 methods, with additional enhancements such of increased test vessel size and extended test duration also trialed. The results have been used to conclude compare the assessment of ready biodegradability across these two protocols, and therefore consider how interchangeably they can be used for environmental risk assessment.

3.05.P-We198 Improved Reliability of Ready Biodegradability Assessment by Omitting Ammonium Chloride from the OECD 301D Test

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The Closed Bottle test (OECD 301D) is a unique test in the OECD 301 series of ready biodegradation tests (RBTs). The low-test substance concentration used in Closed Bottle tests compared to other RBTs makes this test suitable for testing substances that are toxic to the inoculum (Gartiser et al, 2017). The Closed Bottle test is however infrequently performed due to its low potency for demonstrating biodegradability (Gartiser et al, 2023).

In RBT media, ammonium chloride is added to provide nitrogen as a macronutrient for the growth of microorganisms that degrade the test substance (OECD, 1992). Ammonium nitrogen that is not needed for growth will be oxidized to nitrate by nitrifying microorganisms, which are not involved in the actual biodegradation of the test substance. Nitrification is known to increase the probability of obtaining erroneous biodegradability data in OECD 301D tests (Richterich and Steber, 1989; Gartiser, et al, 2023). In addition, nitrification in the Closed Bottle test will increase the background respiration. The background respiration is measured in the inoculum blank bottles and is used as a validity criterion to ensure the accuracy of the test. Compared to other RBTs, the contribution of the nitrification to the maximum allowed background respiration is the highest in the OECD 301D test.

In our research, we performed OECD 301D tests with and without the prescribed addition of ammonium chloride to the test medium. The biodegradability of various test substances with and without organic nitrogen was evaluated, including some difficult to test substances (toxic to the inoculum). River water and activated sludge were used as inocula. At the end of the tests, nitrate and nitrite analyses were performed to correct the biodegradation for the nitrification.

Our research clearly demonstrates that ammonium chloride can be omitted from the OECD 301D test medium without creating a nitrogen deficiency for microbial growth and without decreasing the stringency of the test. The addition of ammonium chloride in the OECD 301D test only increases the chance of exceeding the background respiration, complicates interpretation, and increases the chance of erroneous results.

In conclusion, the reliability of the biodegradability assessment in the OECD 301D test is improved by omitting the ammonium chloride from the mineral salts medium. This modification should be included in future updates of the OECD 301D test guideline.

3.05.P-We199 Correlation of Results from Biodegradation Screening Studies with Physical Properties of the Test Substances

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This work is based on the hypothesis that results obtained from biodegradability screening studies correlate with the intrinsic physical substance properties water solubility and octanol-water partition coefficient (log K_{ow}). Poor water solubility, high log K_{ow} or volatility of the test substances may thwart reaching the pass level in a ready biodegradability test, while with higher solubility or lower log K_{ow}, a higher proportion of readily degradable substances could statistically be expected. Accordingly, the

objective of this work is the statistical evaluation and assessment of such correlations.

As a source of information, the data base OECD eChemPortal was used. The datasets per endpoint as retrieved were filtered regarding several qualitative and quantitative criteria. Further subsets of above basic datasets were formed.

The statistical analysis of the datasets applying Spearman's rank correlation confirmed the initial hypothesis for biodegradation results and water solubility ($r = 2.41$ for basic dataset, 0.07 to 4.78 for subsets) as well as for the correlation with log Kow ($r = -1.93$ for basic dataset, 0.55 to -1.71 for subsets).

This implies that substances exhibiting poor bioavailability have a lower chance of being classified as readily biodegradable or reaching the pass level. In consequence, a substance failing the test is potentially persistent and the next level of testing, e.g., simulation testing, should be considered, or the test result is a false-negative one owing to poor bioavailability.

From the datasets investigated, the most suitable guidelines for biodegradation of poorly soluble and adsorbing substances were derived.

The results obtained suggest that it is worthwhile to aim for avoiding over-conservative or false-negative results from biodegradability screening tests by a well-devised study design, i.e., choosing the test guideline offering the best conditions for poorly soluble and/or adsorbing substances in practice, applying bioavailability improvement methods, and/or using enhancements.

3.05.P-We200 Biodegradability Screening (OECD 301: Test of Ready-Biodegradability) of Several Poorly Water-Soluble Substances

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Biodegradation is an important environmental process that transforms chemicals into simpler compounds and can lead to mineralization or incorporation into biomass of microorganisms. This process is essential for mitigating potential impacts of chemical pollution and supporting sustainable practices. The assessment of biodegradation under the EU REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) regulation typically begins with a stringent screening test for ready biodegradability, using the OECD 301 test guidelines. These tests screen for the potential for rapid mineralisation of substances across various environmental compartments including water, soil, and sediment. However, as the OECD 301 test is an aquatic system, poorly soluble chemicals may be difficult to dose into the water phase, which can lead to misleading results regarding the chemical's inherent biodegradability. The biodegradation of poorly soluble substances is likely hindered due to their limited bioavailability to microorganisms in the aquatic media, as high test concentrations are required (in general, 3-100 mg/L).

There are several bioavailability improvement methods that can increase the chemical-microorganism interaction, such as using solvents and solid substrates, listed in the ISO guideline ISO 10634. In this study we tested seven poorly water-soluble substances to understand their biodegradability in the OECD 301F test using five different bioavailability improvements: specifically ultrasonic dispersion, adsorption onto silica gel, silicone oil and heating with direct addition. Additional guidance from ISO 10634 and the ECHA endpoint specific guidance document Chapter R.7b (Version 4, 2017), led to the inclusion of five additional control groups for poorly soluble substances. This project showed that several of the poorly soluble substances were readily biodegradable (>60% mineralization in 28 days) when appropriate bioavailability improvements were used compared to direct addition. It was also observed that efficacies of bioavailability improvements can differ even for substances with similar structures. Results from this study highlight several issues in biodegradation testing of insoluble substances such as the choice of appropriate bioavailability improvement, the number and performance of control groups required under regulatory guidance, and the most appropriate strategies moving forward.

3.05.P-We201 Improved Interpretation of Ready Biodegradability Tests for Tire-Derived Chemicals: Insights into Microbial Community Structure and Biodegradation Profiles

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Tires are a significant source of environmental contaminants, releasing complex chemical mixtures into ecosystems via physical abrasion and chemical leaching. Despite their widespread presence across environmental matrices, including freshwater and wastewater treatment plants (WWTPs), the environmental fate and persistence of tire-derived chemicals remain poorly understood. Ready

biodegradability tests (RBTs) serve as critical screening tools to evaluate whether these chemicals are rapidly removed or potentially persistent in the environment. However, RBTs often exhibit high variability across replicates, test conditions, and facilities, which is in parts influenced by the microbiota in the environmental inoculum. The presence, abundance, and activity of microorganisms with biodegradation potential can affect both lag phases and biodegradation kinetics. The environmental microbial community involved in these tests often remain as a black box, despite its central role in biodegradation outcomes. This study aims to enhance the interpretation of RBTs by investigating the biodegradability of 11 representative tire-derived chemicals, including 6PPD, diphenylguanidine, benzothiazoles, and benzotriazoles, while simultaneously characterizing microbial community composition via 16S rRNA gene amplicon sequencing. Key variables explored include (1) test concentration, (2) inoculum source (freshwater and WWTP), (3) inoculum toxicity, and (4) structure-biodegradability relationships. Initial findings revealed that none of the tire chemicals tested were readily biodegradable; however, five were classified as ultimately or inherently biodegradable. Notably, the widely used and highly toxic antioxidant 6PPD demonstrated mineralization in some replicates at high exposure levels (15 mg/L). In contrast, no biodegradation was observed for benzothiazole, hydroxybenzothiazole, and mercaptobenzothiazole at similar concentrations, but reducing the concentration fourfold shortened lag phases and increased biodegradation rates. Our results underline the importance of microbial community monitoring in understanding biodegradation profiles of tire-derived chemicals. These insights are critical to advancing the information output of RBTs and supporting environmental risk assessments of tire-related contaminants.

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3.05.P-We202 Overcoming Challenges and Advancing (Bio)degradation Guidelines: OECD TG 309 Revisited

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In Europe, if a chemical is non-readily biodegradable, and is manufactured or imported in quantities of 100 tonnes or more, biodegradation simulation testing is required under the REACH regulation. Simulation testing may also be requested for lower tonnage substances, if a concern for PBT/vPvB properties has been flagged. The OECD 309 Test Guideline (Aerobic Mineralisation in Surface Water), which was last updated in April 2004, hence plays a pivotal role in assessing the environmental fate of chemicals globally. Over the last 20 years, the state of science has evolved, and updates to the OECD 309 Test Guideline are anticipated. ECETOC organized a 2-day workshop in January in Brussels, bringing together participants from regulatory bodies, academia, industry and environmental non-governmental organisations (NGO), having expertise in biodegradation testing and persistence assessment. The objectives of the workshop included to (1) discuss and address current challenges and limitations in conducting and interpreting degradation simulation studies, (2) define/align on the key updates needed for the OECD 309 test guideline, (3) define a roadmap to ensure that the prioritised updates flagged would be addressed in time for the OECD 309 test guideline update, and (4) identify research needs and potential methodological advancements to improve the guideline's relevance and applicability, with possible implementation in other test guidelines. This was coordinated in multiple break out discussions focussing on robustness, interpretation and relevance. The outcomes of the workshop presented in this poster highlight further research needs that the community can address to improve biodegradation and persistence assessment.

3.05.P-We203 Results of Ring Testing for the Selection Reference Substances for Improving Chemical Persistence Assessment in Higher Tier OECD 309 Simulation Test

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The OECD 309 test guideline is a crucial tool for assessing the biodegradation and persistence of organic contaminants in surface water. However, variability in test parameters and inoculum characteristics can lead to inconsistencies in observed degradation kinetics. The inclusion of substances with varying

biodegradation profiles will provide a more comprehensive benchmark for assessing inoculum activity and overall test performance.

A ring trial has been performed, focusing on two selected candidate reference substances: caffeine and 2,4-D. These substances were chosen from an initial pool of 32 candidates, which was narrowed down to eight based on relevant properties, and availability of radiolabelled forms of the compounds. Further screening using a modified OECD 301D study led to the final selection of caffeine and 2,4-D, alongside the existing reference substance aniline. Caffeine, known for its ready biodegradability with a lag phase, and 2,4-D, characterized by slow or incomplete biodegradation, were subjected to rigorous simulation testing. The tests were conducted under various conditions, including two temperatures (12°C and 20°C), two concentrations (1 and 10 µg/L), and using inoculum from two water sampling locations. This comprehensive approach aimed to capture each substance's biodegradation profiles and assess their performance under diverse environmental conditions.

The interlaboratory ring trial aimed to assess the suitability of the selected candidate reference substances and to evaluate test validity across different laboratories. Nine laboratories from different parts of Europe participated in the test. Involving multiple laboratories enabled the assessment of reproducibility and consistency of OECD 309 test results when using the proposed new reference substances. This collaborative effort sought to enhance the reliability of OECD 309 testing by providing a more comprehensive benchmark for assessing inoculum activity and overall test performance. The inclusion of substances with varying biodegradation profiles aimed to capture a broader range of degradation behaviours, potentially improving the test's ability to assess the persistence of diverse organic contaminants.

By conducting this extensive interlaboratory study, the project has aimed to strengthen the foundation of OECD 309 testing, ultimately supporting more accurate and consistent persistence assessments for regulatory purposes in environmental risk evaluations.

3.05.P-We204 OECD 309-Aerobic Mineralisation in Surface Water-Results from a Laboratory Participating in the CEFIC-LRI ECO55 Ring Trial

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The OECD 309 guideline is the aerobic mineralisation in surface water simulation biodegradation test. The purpose of the test is to assess the biodegradation and mineralisation of test items in natural water at low concentrations. These studies are usually conducted with radiolabeled test items at test concentrations between 1 and 100 µg/L. The use of radiolabeled test item allows primary degradation and mineralisation to be assessed and mass balances to be measured. The identity of primary degradates can also be investigated.

As part of the test a reference substance is used to ensure that the natural water contains an active microbial population. Historically either aniline or sodium benzoate have been used and it is expected that they will be substantially degraded within two weeks.

Under the European Chemicals Agency's (ECHA) regulations for the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), the OECD 309 test has become the favoured test to assess persistence in the environment for industrial chemicals at 12°C (freshwater).

It is considered that aniline and sodium benzoate do not provide a sufficiently robust assessment of the viability of the natural water as they are too easily mineralised to carbon dioxide. Therefore, as part of the CEFIC LRI funded project, ECO55, investigating the parameters around the OECD 309 test, the use of alternative reference substances was proposed. The alternative reference substances investigated in this interlaboratory ring trial are caffeine and 2,4-dichlorophenoxyacetic acid.

The objective of the ring trial is to identify a reference substance which will better assess the viability of the test water and therefore lead to an improved assessment of persistence. The overall aim of the project is to update the guideline to provide a more consistent and robust measure of persistence for chemicals in the environment.

The poster will present the data generated in tests conducted, using each of the reference substances, in our laboratory and compare the results we obtained to those for aniline and sodium benzoate.

3.05.P-We205 Investigation into Reference Substance Degradation Rates in OECD 309 Tests

Desmond Kelly and Daniel T Evans, Smithers ERS, United Kingdom

Simulation tests performed using a radiolabelled molecule are important in determining the primary and ultimate degradation of a chemical and evaluating its potential to persist in the environment. The surface water mineralisation test performed according to OECD test guideline 309 incorporates a reference substance as a positive control to demonstrate that the water contains an active microbial population. The

test guideline suggests aniline or sodium benzoate as the reference substance and that the expected time interval for degradation is usually less than two weeks.

The current work was undertaken to understand the expected rate of degradation of these reference substances when incubated under varying conditions. Surface water with suspended solids in the range 10–20 mg dry weight/L was freshly collected and treated with ¹⁴C-aniline or ¹⁴C-sodium benzoate at a rate of 10 µg/L. The treated samples were then incubated under aerobic conditions at 12 and 20°C, and the rate of mineralisation to ¹⁴CO₂ was monitored at regular intervals throughout the incubation period. Further investigations were performed using surface water from different sources. In addition the impact of different amounts of suspended solids was assessed.

The results of the work are discussed in this presentation.

3.05.P-We206 Will Carbon Capture Plants Contaminate Drinking Water? Biodegradation Potential of Nitramines in Lake Water

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There is a potential risk associated with (potentially) carcinogenic nitrosamines (NSAs) and nitramines (NAs) that can form in the air from amines escaping amine-based carbon capture plants (CCP). These are very soluble molecules that can end up in drinking water sources such as lakes and groundwater basins. In Norway, a drinking water threshold of 4 ng/L has been set for the sum of NSAs and NAs. A dynamic modelling tool is under development to guide the industry and regulators to ensure that the safety limit will not be exceeded. In the model, the major removal mechanism for NSAs is photodegradation, while biodegradation is the dominant removal mechanisms for NAs. Thus, reliable biodegradation rates for NAs are needed to realistically simulate future levels in the water compartment.

A modified OECD 309 simulation test for biodegradation in surface water was used to assess biodegradation rates of three representative nitramines. Caffeine was used as positive reference control. The study site was a drinking water lake located in the vicinity of a planned CO₂ capture plant. Water and sediment were collected at different seasons, and the tests were performed with and without added sediment. The three nitramines were added either separately or together to the test bottles, and primary degradation was measured by LC-MS/MS compared to the abiotic control. The biodegradation rates varied between experiments. However, the difference in degradation rates was consistent with (monoethanol)-nitramine (MEA) being the most rapidly degradable, 2-methyl-2-(nitramine)-1-propanol (AMP) being moderately degradable and N-nitropiperazine (NIPZ) having limited degradation after 60 days. Adding sediment to the surface water increased biodegradation rates. The effect of different modifications of the test set up (with/without sediment and single vs mixture of test substance) will be presented and discussed.

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3.05.P-We207 The Influence of Spike Timing and Inoculum Stability on the Biodegradation of Organic Compounds in a Water-Sediment Test System

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The OECD 309 test is a widely accepted method for determining the biodegradation rates of organic substances utilizing batch incubations with pelagic or suspended sediments. While it is a highly recommended biodegradation test, it faces challenges from inconsistencies and variable results, which require further investigation. Also, our understanding of the inoculum, specifically the microbial community dynamics in environmental samples during simulated tests, remains limited. This includes evaluating microbial stability and examining critical aspects of batch incubation experiments, e.g., test compound spiking, which may influence microbial diversity and community composition. In particular, the period between contaminant spiking can influence whether microbial communities acclimate to the contaminant, potentially increasing the degradation rate in subsequent exposures. Spike timing refers to the point at which test substances (i.e., contaminant mixtures) are introduced to the test system, and it is crucial as it determines the inoculum's exposure to the substance, influencing the observed biodegradation rates. These insights could be instrumental in advancing the standardization of simulated biodegradation testing. Here, we profiled the diversity and composition of the batch incubation microbiome at different spiking times and linked this to the biodegradation capacity of the inoculum against 129 test compounds. In particular, we sampled a wastewater-impacted river segment, followed the OECD 309 test guidelines

for sediment-water collection and preparation, and spiked the samples at different time points, i.e., 0 hr, after 24 hrs, 2 days, and 3 days. We used 16S rRNA amplicon sequencing to profile the microbial communities' taxonomic and putative functional information. Our observations will be useful in improving our knowledge of the microbial community dynamics in biodegradation tests. This will also provide insights into the environmental relevance of sediment microbiome samples used for standard simulation tests.

3.05.P-We208 Challenges and Successes in Radiolabeling and Characterizing Polymers for Environmental Simulation Studies

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The ability to quantify the rate and extent of chemical biodegradation using laboratory simulation studies that mimic real world conditions is important for an accurate evaluation of environmental fate. Simulating environmental conditions requires tests to be conducted in complex environmental matrices under realistic test concentrations which makes analytical sensitivity a challenge. Radiolabeled test materials are a well-accepted solution. Synthesis of radiolabeled polymers and analytical characterization of the parent and degradation metabolites provide new technical challenges as the methods traditionally employed for small molecules are not all applicable to polymers due to their typically higher molecular weight and complex multi-component structures. In this poster we will discuss a variety of isotopes (³H, ¹⁴C) and synthesis methods (specific, general, and uniform) currently employed to generate radiolabeled polymers. Further, analytical approaches (thin layer chromatography, gel permeation chromatography, nuclear magnetic resonance, and molecular weight cut off filters) used for the characterization of radiolabeled polymers and metabolites will be discussed. The data presented will support the assertion that both biobased and synthetic polymers can be radiolabeled and characterized while highlighting technical watch outs and challenges.

3.05.P-We209 Polymer Biodegradability and the Link Between Abiotic and Biotic Degradation

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Polymer persistence is a growing environmental concern, and current biodegradability tests are often designed for small molecules and may not adequately capture the complexities of polymer degradation. This study evaluates the impact of hydrothermal pre-treatment on polyester biodegradability using a novel assessment method combining pre-treatment and advanced physicochemical and chemical analyses in parallel to biodegradability testing, enabling the identification and monitoring of specific degradation products.

Polyhydroxybutyrate (PHB) and polylactic acid (PLA) served as model biodegradable and non-biodegradable polymers, respectively. Hydrothermal ageing (130°C) was performed for varying durations (1-120 hours). Following hydrothermal ageing, water-soluble degradation products were analyzed directly by Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) to characterize the initial breakdown products. Subsequently, OECD 301F biodegradability tests were conducted on pristine and aged PHB and PLA particles, extracted water-soluble oligomers, and the corresponding monomers. To analyze the degradation products formed during these biodegradability tests, Solid Phase Extraction (SPE) cartridges were employed for efficient extraction from the complex biodegradability medium before LC-HRMS analysis.

The analysis confirmed the presence of PHB oligomers of up to 5 repetition units in hydrothermally aged samples. These same oligomers were identified at a strategically chosen 5th day of the biodegradation test. Hydrothermally aged PHB showed enhanced biodegradability (shorter lag phase, faster 60% threshold achievement), while PLA remained non-biodegradable. The contrasting responses of PHB and PLA suggest a possible correlation between the extent of hydrothermal degradation and biodegradability. This finding indicates a potential rapid screening test methodology for polyesters, which will be further investigated with additional biodegradable polymers upon solubilization/improved bioavailability such as polycaprolactone (PCL). This improved assessment approach advances our understanding of polymer environmental fate.

3.05.P-We210 Identifying Bound Compounds in Non-Extractable Residues of Pesticides in Soil by 4-Pool Kinetic Analysis

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Non-extractable residues (NERs) of pesticide in soil are one of the major uncertainties in pesticide risk

assessment and persistence evaluation for PBT and PMT classification, because of their unknown chemical identities. This study explores the feasibility of identifying bound compounds in non-extractable residues (NERs) of pesticides in soil by 4-pool kinetic analysis. The 4-pools refer to parent compound, metabolites, NERs, and CO₂ in ¹⁴C-labeled pesticide soil degradation studies. We discovered the following two characteristic 4-pool kinetic behaviors of formation of NERs: (1) if parent compound is bound as NERs, the metabolites (m(t) in % applied radioactivity (AR)) kinetically drive the evolution of CO₂ only; and (2) if a metabolite (x) in a sequential degradation pathway is bound as NERs, m(t) is split into m₁(t) and m₂(t) at the metabolite (x) that is bound as NERs, which kinetically drive the formation of NERs and evolution of CO₂ respectively. We developed two (i.e., Parent->NER and metabolite->NER) 4-pool models to capture the kinetic behaviors respectively. By fitting the models to a set of 4-pool data, we not only determine whether NERs form from parent compound or metabolites but also identify the bound metabolite (x) by resolving the metabolites into M₁(t) and M₂(t) (i.e., the variables used to simulate m₁(t) and m₂(t)) and then matching m₁(t) (i.e., the sum of bound metabolite (x) and its metabolite precursors) with M₁(t). By applying the models to ¹⁴C-labeled parent compound-dosed studies and then sequentially to metabolite-dosed studies for 7 pesticides with ~70% AR NERs, we identified the bound compounds, which have the moieties known to be responsible for NERs, are bound as NERs instantaneously when dosed into soil, and account for pH dependency of NER formation. This study demonstrated that we could identify the bound chemical species in NERs of pesticides, assess the risk/hazards of NERs at a molecular level, and thereby eliminate the uncertainties in pesticide risk and persistence assessments.

3.05.P-We211 Developing Principles for Read-Across of Biodegradation Simulation Test Endpoints

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Biodegradation simulation tests are a standard information requirement under the EU REACH regulation and aim to provide information on the degradation rate of substances and the formation of transformation products under realistic environmental conditions. These tests are complex and costly and carry important implications for the evaluation and risk management of substances. In practice, few substances registered under REACH currently have simulation testing data available. In the absence of data, it is possible to address standard information requirements using adaptations. One such adaptation is read-across, whereby data for one or more substances (source substance(s)) are used to support the data requirements of other substance(s) (target substance(s)). This can be advantageous for reducing the costs of meeting information requirements, and for increasing the efficiency of the evaluation process.

Read-across is an established approach for (eco)toxicity endpoints. However, the application of read-across to degradation endpoints has so far received little attention. In this poster, the concept and principles of read-across will be explored and applied to the specific information requirements of biodegradation simulation tests. The regulatory requirements for justification of read-across will be discussed, including the ECHA read-across assessment framework (RAAF) and how this might apply to biodegradation simulation test endpoints.

Simulation tests can produce information on both the degradation half-life of the substance and the identity of transformation products. Read-across approaches could consider one, or both, of these aspects, and each may need to be justified separately. For example, two substances may have a similar degradation half-life but different transformation products, or vice versa. Potential considerations related to assessing the suitability of read-across for both aspects will be discussed (such as considering the behaviour of source and target substances with respect to non-extractable residue (NER) formation). Further, the potential use of a data matrix, as discussed in RAAF, to support a category approach for reading across simulation test endpoints will be presented.

3.05.P-We212 Biochemical Oxygen Demand as a Proxy for Micropollutant Biodegradation

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The biodegradation rate of organic micropollutants in the environment is highly variable, presenting a challenge when assessing their risks and the exposure of humans and ecosystems. Simultaneously, measuring these biodegradation rates for a given site using established methods such as the OECD 309 is both time consuming and expensive, limiting the information available to researchers and regulators. It would therefore be useful to develop a faster and more affordable test which provides an indication of environmental biodegradation rates, allowing a large number of sites to be screened using less time and resources. In this study, we assessed if a modified three-day Biochemical Oxygen Demand (BOD₃) test could serve this purpose. This was done by applying a modified BOD₃ test in parallel with a modified OECD 309 test to water and sediment samples, simultaneously assessing BOD₃ and the first order

attenuation rate constant for 47 spiked micropollutants. Water and sediment samples were collected at 10 sites in the Fyris river and its tributaries in Uppsala, Sweden. Attenuation rate constants for at least three sites were obtained for 22 spiked compounds, allowing the relation between the two metrics to be assessed using linear least squares regression. There was a strong positive correlation ($r^2 > 0.8$) between site BOD3 and attenuation rate for 12 of the 22 compounds, and a moderate correlation ($r^2 > 0.5$) for a further 5 compounds. For the last 5 compounds there was no clear correlation between attenuation rate and BOD3. The results reveal that a modified BOD test could have the potential to serve as a screening tool for the micropollutant biodegradation capacity of aquatic systems. Before broad application, it is however necessary to test the relationship between the two metrics for samples from different river systems.

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3.05.P-We213 Towards Streamlined Environmental Persistence Assays for Trace Organic Contaminants: Findings from High-Throughput Method Optimization and Biodegradation Testing
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The 2022 European Commission framework entitled Safe and Sustainable by Design chemicals and materials emphasizes the need for early assessment of the hazardous properties of chemicals during their design. One such property is environmental persistence, which is currently evaluated using outdated, time-intensive, and resource-heavy methods. To address this, we developed a high-throughput method for evaluating chemical persistence using activated sludge as a microbial inoculum source. Performing the assay in activated sludge allows for a rapid (48 hour) assessment of pollutant biodegradation, while avoiding the microbial lottery and still allowing for extrapolation of the results to environmental compartments such as soil. Our approach combines a 24-well plate format with an eight-minute reverse-phase liquid chromatography runtime and high-resolution mass spectrometry for the high-throughput detection of micropollutants. The method was developed based on a test set of 40 compounds, primarily pesticides, and demonstrated comparable half-lives between the 24-well plate and traditional large-volume biotransformation experiments. To further assess the method's throughput and applicability domain, we applied it to a mixture of 200 compounds including pharmaceuticals and agrochemicals and analysed their degradation and adsorption behaviour in the miniaturized setup compared to large volume reference data. The presented results will include comparisons of persistence between the large-volume and miniaturized formats for the 200 compounds, as well as an investigation into the impact of chemical mixtures and dissolved organic carbon (DOC) content on degradation behavior. We hypothesize that minimal mixture effects will be observed due to the environmentally relevant spike concentrations and based on preliminary experiments with the initial test set of 40 compounds. However, the DOC introduced by the solvent used to dissolve the standards may influence biotransformation rates, as these spikes significantly elevate the carbon content in the samples and provide an easily accessible carbon source for microorganisms. By developing a high-throughput screening method for measuring primary biotransformation, a first-tier assessment can be made on the persistence of chemicals, which is especially useful for early-stage chemical development pipelines.

3.05.P-We214 Persistence Directed Testing of potential PMT Chemicals in Mixtures

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Environmental persistence is the critical property of organic pollutants that makes them remain in the environment for extended periods of time and notoriously difficult to remove again. The current persistence assessment strategy is based on the biodegradation testing of individual chemicals with the implicit aim to demonstrate biodegradability rather than persistence. This biodegradation directed testing of each chemical is time consuming, and many chemicals have consequently not yet been tested. Persistent chemicals that are still untested and therefore remain unregulated might cause severe and long-lasting pollution problems. Therefore, there is a need to turn the test strategy upside down and focus on finding and managing the most persistent chemicals.

We propose persistence directed aquatic testing of chemicals conducted in mixtures, where the persistence of each chemical is assessed based on substrate depletion in biotic test systems (incubated with surface water) relative to abiotic controls (i.e., absence of primary degradation). The approach can speed up biodegradation testing, since one test can yield biodegradation data for e.g., 20-50 chemicals and is particularly suited to uncover the most persistent chemicals in the mixture. In the current project we will adjust and apply this approach to develop a time-efficient and reliable persistence directed testing strategy to investigate suspected PMT and vPvM chemicals.

This project poster introduces a novel tournament test strategy to narrow a larger number of potentially persistent chemicals down to a smaller number of confirmed persistent chemicals through three rounds of persistence testing. The absence of primary degradation in all rounds will be used as sound evidence of persistence (P and vP). At least 3 inoculums will be used to maximize the reliability of the conclusions. A small number of test chemicals will be included in all mixtures and serve as reference substances and benchmark substances. Final biodegradation tests will be conducted with single substances to increase regulatory acceptability.

3.05.P-We215 Assessing Persistence in Aquatic and Aquatic Sediment Systems using Static and Semi-Continuous Biodegradation Test Systems

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The persistence of chemicals in the environment is now the most critical parameter in regulatory frameworks for environmental hazard assessment. The extension of the hazard criteria from persistent, bioaccumulative and toxic substances (PBT) to include persistent, mobile and toxic substances (PMT); and their very persistent and very bioaccumulative (vPvB), and very persistent and very mobile (vPvM) categories of increased concern, coupled with stakeholder concerns about the continued presence of chemicals in the environment make persistence the key property of concern in hazard assessment. Scientific innovations to capture environmental realism and relevance in chemical persistence assessments are lagging the regulatory frameworks aimed at restricting the use of hazardous chemicals. There is an overreliance on biodegradability assessments that have limited microbial ecological and functional relevance due to their limited biomass concentrations, biomass preconditioning regimes, the batch nature of the study, limited test volumes and limited test durations. There is also a mis-placed perception that persistence is an intrinsic substance property amongst some communities; whilst some chemical structures will be inherently more persistent than others this premise fails to allow for microorganisms to adapt and evolve new catabolic capabilities over time. These dated and misplaced perceptions are leading to the over-classification of chemical persistence and hindering chemical innovation. Within this presentation we will demonstrate that lag periods and adaptation times often exceed the test duration of many standard biodegradation test guideline studies (e.g., OECD 301 and 309) before the onset of rapid biodegradation for chemicals widely considered to be non-persistent (e.g., nitrophenols, anilines and chloro-anilines). The presentation will also demonstrate that (1) the presence of settled or suspended sediment within the test system can reduce lag periods, and (2) that or semi-continuous operation demonstrates reductions in lag periods overtime with increased biodegradation rates as microbial communities adapt.

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3.05.P-We216 Biodegradation Testing Strategies in the Framework of REACH and CLP Regulations

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Biodegradation assessment of chemicals plays a key role to protect human health and ecosystems in EU regulations (REACH regulation EC n°1907/2006 and CLP regulation EC n°1272/2008), especially with the recent implementation of the latest Persistent, Bioaccumulable, Toxic (PBT)/very Persistent and very Bioaccumulable (vPvB) and Persistent, Mobile, Toxic (PMT)/very Persistent and very Mobile (vPvM) hazard classes in CLP regulation.

Based on current biodegradation tests, a chemical substance can be considered as readily biodegradable (with or without the 10-day window), inherently biodegradable (fulfilling or not specific criteria) or not biodegradable in a REACH registration dossier.

The aim of this work is to show how different results of biodegradation tests can have an impact on testing strategies for REACH requirements and for CLP regulation depending on the registration tonnage band. Based on comparison of stepwise approach developed by ECHA to assess biodegradation/persistence and recent ECHA reviews of REACH dossiers, a flowchart has been created to identify the requirement(s) depending on the result(s) of biodegradation tests.

Only chemical substances considered as readily biodegradable or inherently biodegradable are not classified persistent according to PBT/vPvB assessment. Despite the fact that a substance is not classified as persistent, further tests on the degradation of the substance can be required to meet the requirements of the REACH regulation and refine the Soil Hazard Category of a substance.

A substance not biodegradable is considered as persistent in different assessment of REACH and CLP (PBT/vPvB, REACH requirements, Soil Hazard Category).

Overall, the results indicate that different interpretations of persistence can be made for the same

substance depending on the EU regulation. Interpretation of biodegradation results for persistence assessment among ECHA guidances should be harmonised in the context of the future one substance-one assessment approach in Europe.

3.05.P-We217 The Persistence Assessment Tool (PAT): Implementing a Methodology for Data Quality Evaluation and Weight of Evidence in Persistence Assessments

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Interest in chemical persistence has increased significantly in recent years, with Persistent, Mobile, and Toxic (PMT) and very Persistent, very Mobile (vPvM) now hazard classes under the CLP Regulation. Regulatory persistence assessments require building a weight-of-evidence (WoE) to compare chemical degradation half-lives to set criteria for different environmental compartments, using all relevant lines of evidence (LoE) e.g., biodegradation screening tests, field data, etc. Implementation challenges remain in persistence assessments, particularly relating to guidance around data quality evaluation and the WoE determination. In addition, there are issues for substances whose properties render them difficult to evaluate using standard methods.

To address these challenges, the freely available Persistence Assessment Tool (PAT) was developed to support the evaluation of persistence and released on the Ricardo website. To develop the PAT, available persistence assessment guidance was extracted and converted into rules and logical tests to be coded into a stepwise methodology. First, the quality of individual studies are evaluated, which are then combined within the same LoE to draw conclusions at the LoE level. Information from different LoEs are then weighted and integrated into single persistence assessment conclusions. This software tool provides clear guidance and structure to evaluate data quality, and a quantitative WoE methodology to process the information and calculate persistence conclusions in line with regulatory guidance. The PAT provides specific features to account for difficult and complex substances and includes various options for customisation to specific regulatory frameworks. In addition, a multimedia fate model, SimpleRisk4PAT, is included to optionally calculate overall persistence (Pov).

Since the PAT is mentioned as a WoE tool in the new Guidance on the Application of the CLP Criteria, it is important to continually upgrade and improve it. Input from users and stakeholders thus far has led to an update of the PAT, which includes allowing more flexibility in study input parameters and treatment of half-lives, fixing software issues, and including free text fields for assessors to add comments. The PAT aims to support robust, consistent, and transparent decision-making for persistence assessments. Further stakeholder input is needed to support consensus-building and uptake of the methodology.

3.05.P-We218 Can an In Silico Model Predict Ready Biodegradability Results While Enhancing Understanding of Degradation Mechanisms?

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Aerobic microbial biodegradation of chemicals is a key environmental process resulting ultimately in the transformation of organic compounds into water and carbon dioxide. The assessment of chemical biodegradation is crucial to limit the release of compounds that may persist in ecosystems. To experimentally determine if a chemical is readily biodegradable, the OECD 301/310 Guidelines set out specific criteria that must be met. The test is not rapid (28-60 days). In the area of the Safe and Sustainable by Design approach, quick and robust ready-biodegradable property predictions is increasingly important. In many applications the risk of persistence of a substance may limit its commercial potential. Thus, limiting the time and cost while providing, in addition, a mechanistic interpretation and uncertainty of the result is advantageous. We developed a Structure Activity Relationship (SAR) model based on machine learning approaches compliant with the five OECD principles for (Q)SAR method validation. First, numerous data performed according to OECD 301/310 Guidelines were retrieved from the literature in order to cover a wide structural chemical space. To maximise reliability, an in depth curation of the database was performed removing studies that were not OECD compliant or dealing with compounds such as multi-constituents, salts, organo-metallics and stereoisomers. Also, substances for which more than one study was available were manually assessed based on expert judgement. Then, data splitting was performed using a clustering algorithm to ensure structural diversity and a balanced distribution across the training, validation, and test sets, while also considering the response variable to achieve better representativity of the target properties. Following the data split, various machine learning modelling approaches were explored such as a support vector machine, random forest and artificial neural network

among others. Different strategies for setting up the descriptors were tested, including generating numerous atomic descriptors and incorporating specific variables identified in the literature that influence biodegradation. This model represents the first version of KREATiS biodegradation model based on machine learning. Future versions will be dedicated to specific OECD 301 (B,C, D, & F) and 310 methods, include salts and organometallic compounds, as well as integrating microbial ecological data to enhance the model's environmental reliability.

3.05.P-We219 Probabilities to Prioritize Potential Persistent Pollutants

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Chemical hazard and risk assessments often use physical-chemical properties to categorize and identify chemicals of concern. In Europe, recent legislation regarding the classification, labelling and packaging (CLP) of consumer products includes new chemical hazard categories, including persistent, mobile, and toxic (PMT) and very persistent and very mobile (vPvM). Persistency is currently assessed using ready biodegradability data and half-lives of the substance in different systems. This is additionally challenging because of the limited availability of data and information in the environment. We combine available datasets available in literature, data from OECD's eChemPortal and QSAR Toolbox and predict ready biodegradability and degradation half-lives using QSAR models such as OPERA and EPI Suite. We combine data, including availability of uncertainty data and errors, and the relative reliability of the source of data using Bayesian analysis methodologies.

In this work we assess the potential persistence of substances from the ZeroPM Global Chemical Inventory, which includes approximately 119,000 SMILES that represent 93,000 substances. We predict the probability that a substance is not persistent, persistent, or very persistent based on the available data. This data can be used as part of a weight of evidence approach when the probability of any one category is very high or to identify substances where further experimental testing and reporting is required because the probability of a substance being not persistent is near 50% and thus the results are inconclusive. We also explore the entire dataset and the cumulative probability scores, to understand how many of the chemicals registered on the global market may be considered potentially persistent or not persistent.

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3.05.P-We220 Screening Assessment of the Fate of Natural Constituents: A Review of Data and Analysis in the Context of More than One Constituent Substances (MOCS)

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Natural Complex Substances (NCS), such as essential oils, are substances of natural origin with a complex chemical composition. They are distinguished by their heterogeneous nature, resulting from biological, geological or physical processes without significant chemical modification. The revision of the CLP regulation, part of the EU Green Deal's Chemical Strategy for Sustainability (CSS), aims to ensure a toxic-free environment by updating classification rules for complex substances referred to as More than One Constituent Substances (MOCS). The Parliament and the Council agreed on a specific derogation for plant extracts not chemically modified, including essential oils, with a 5-year review of scientific evidence by the Commission. Last year, the update of the CLP, regulation (EU) 2023/707, introduced PMT/vPvM and PBT/vPvB as new hazard class and criteria.

For this scientific challenge within the framework of NCS, it is essential, first of all, to assess the environmental fate (Persistence, Bioaccumulation, Mobility) and environmental Toxicity (PMBT) potential of the major constituents present in commercial essential oils.

Starting from a list of essential oils in the market and their composition, we have analysed over 1,000 constituents. A screening PBMT assessment has been conducted using the outcome of various QSAR models (e.g., BIOWIN and CATALOGIC for biodegradation/persistence, KOWIN, BCFBAFWIN and CATALOGIC for bioaccumulation, KOCWIN for mobility, ECOSAR and iSafeRat for ecotoxicity) and when available experimental data. These later were extracted from the QSAR ToolBox v.4.7 and from the Research Institute Fragrance Materials database. This allows us to compare the predictivity potential of the various models. In a second phase, the analysis will focus on the high-impact constituents, i.e., less than 100 constituents representing around 99% of the total volumes of constituents.

This study offers a unique perspective on the environmental fate and ecotoxicity of a large number of natural constituents present in essential oils. Focusing on the available experimental data and supplementing it with our reliable predictions from models will highlight the datagaps for those

constituents. This analysis provides an initial information on constituents that will support discussion about the application of the MOCS rule to plant extracts that have been chemically modified.

3.05.P-We221 Lost in Complexity: Limitations of the Current REACH Guidance in Assessing UVCB Persistence

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The REACH regulation encounters significant challenges when assessing complex substances like UVCBs. These mixtures are characterized by intricate compositions, often consisting of hundreds of isomers, many of which may be unknown or unidentified. This complexity poses substantial difficulties in evaluating their persistence in the environment.

One primary issue is the impossibility to analytically determine a main component within these mixtures. The diversity of isomers makes isolating a single compound for targeted testing nearly impossible. Consequently, methodologies such as radioactive labeling, which could facilitate degradation studies through simulations, become unfeasible. Without a clear understanding of specific components, traditional persistence testing methods may yield unreliable or misleading results.

Moreover, the current ECHA Guidance is often too generic and vague to effectively address the unique challenges posed by UVCBs. Established protocols typically overlook the complexity of these substances, leading to a one-size-fits-all approach that fails to capture their intricacies. This limitation is further exemplified by the use of QSAR modeling. While QSAR can be a powerful tool for predicting the behavior of chemicals, applying it to individual isomers of UVCBs can be of little value under certain circumstances. The uncertainty of these modeled structures in the UVCB means that any predictive outcomes may not accurately reflect reality.

In our specific case, a screening study of the entire UVCB resulted in >97% degradation, providing the most informative assessment of the biodegradation behavior of this specific UVCB. While this approach may lack the precision of more focused analytical methods, it generates a holistic understanding of the environmental fate of the substance, considering interactions among its various isomers. However, this method may not be universally applicable to all UVCBs, as each substance presents unique challenges that require tailored assessment strategies.

In conclusion, the existing REACH guidance is inadequate for assessing the persistence of complex UVCBs. The multitude of isomers, the challenges in identifying a main component, and the limitations of standard methodologies highlight the need for more tailored strategies. As the understanding of UVCBs continues to evolve, regulatory frameworks must adapt to develop specific guidance that addresses the complexities of these substances more effectively.

3.05.P-We222 Higher Tier Biodegradation Assessments of Industrial UVCBs: Which Alternative Approaches are Acceptable?

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A case study is presented in which the (environmental) degradation products of a complex industrial UVCB compound were predicted using the online rule-based model EAWAG-BBD Pathway Prediction System, as performing higher tier experimental biodegradation testing was deemed technically infeasible. The aim was to obtain a list of predicted (bio)degradation products (PBPs) that may form in the environment and then determine their persistency, bioaccumulation and toxicity (PBT/vPvB) profile. The parent compound consisted of 3 different main constituents, each constituent itself present as a mixture of structures varying in length. However, the substance's complexity and the conservative nature of the computer model caused unexpected issues for the alternative approach as well, as a list of roughly 80,000 unique PBPs was generated. This occurred despite that the substance's constituents were deemed to fall within the model's domain of applicability. Attempts to group the PBPs were abandoned due to too much overlap and the need to justify the use of groups/categories. Hence, the PBT/vPvB characteristics of all the predicted PBPs were determined using different models of EPI Suite (US EPA).

The results of the PBT/vPvB assessment indicated that whereas a majority of the PBPs was predicted to be not readily biodegradable, none of them should be considered as potential PBT/vPvB substances. No potential for bioaccumulation was observed and the estimated toxicity for aquatic organisms was predicted to be low. In addition, only minor issues with model domain applicability were observed for a minority of the PBPs, none of which were deemed to impact the final conclusion of the assessment.

Still, despite that using experimental studies is often technically not possible for substances like this, regulatory acceptance of alternative approaches, regardless of how conservative their results might be, remains of concern. This is due to the apparent expectation of the regulator that alternative approaches

should provide the exact same type of information as the experimental studies they replace. As such alternative approaches are currently lacking, there is currently not enough clarity on how to approach the biodegradation assessment of industrial UVCBs such as the one used in this case study. This presentation aims to open and contribute to this discussion.

3.05.P-We223 Navigating the Complexities of Biodegradation Testing for UVCBs: Challenges, Approaches, and Case-study Insights

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Substances of Unknown or Variable Composition, Complex Reaction Products, or Biological Materials (UVCBs) represent a significant challenge in environmental risk assessment due to their structural and compositional complexity. This study highlights the methodological approaches, challenges and potential solutions in assessing the persistence of UVCBs, using a case study to demonstrate practical insights. There are 3 main approaches recommended to test UVCBs, including the known constituent approach, block profiling, and the whole substance approach. Each strategy offers advantages depending on the availability of constituent-specific data and analytical feasibility. In a case study, the biodegradation of a UVCB substance was investigated using the known constituent approach. A representative component (the test item) was selected from the UVCB substance, followed by radiosynthesis and biodegradation testing in surface water according to OECD 309 test guideline. Results indicated significant degradation differences between low (10 µg/L) and high (100 µg/L) concentrations, with maximal biodegradation reaching 56 % and 0.4 %, respectively, after 60 days incubation. Apart from significant difference in the observed degradation rate for high and low concentrations, differences were also observed in the biodegradation variability between replicates at later time points, pointing to potential differences in microbial diversity and density.

Key challenges for biodegradation testing were identified based on the results including divergent degradation rates across replicates, concentration-dependent discrepancies, and radioactivity losses during sample processing. Addressing these challenges require enhanced microbial monitoring, better reference substances, and the integration of new approach methodologies (NAMs) and QSAR models.

This study underscores the importance of tailored testing approaches, the integration of microbial insights, and methodological refinement to advance our understanding of UVCB biodegradation. Furthermore, the study demonstrates that for UVCB-Substances, such method is very expensive and becomes economically unsustainable when multiple components of the UVCB-substance need to be examined.

3.05.P-We224 Organic Micropollutant Biodegradation Capacity of Dutch Drinking Water Aquifers

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Water quality in Dutch drinking water aquifers is threatened by the increasing presence of organic micropollutants (OMPs) such as pharmaceuticals, pesticides and household chemicals. Aquifers are oligotrophic and anaerobic environments where the ability of indigenous microbial communities to biodegrade OMPs is limited. Supplying oxygen and a biodegradable carbon source harvests the OMP biodegradation capacity present in aquifers. Biodegradation capacity is shaped by the geochemical characteristics of aquifers, leading to variations in biodegradation rate and removal efficiency of specific OMPs. This variation indicates that assessing the potential of Dutch drinking water aquifers to support OMP biodegradation requires further investigation. This research aims to survey conditions determining the OMP biodegradation capacity of Dutch drinking water aquifers and clarify the role of biodegradable dissolved organic carbon (DOC) in this process. The biodegradation capacity of more than 20 OMPs was tested at environmental concentrations (1 µg/L) during aerobic batch bottle experiments inoculated with indigenous microbial communities from 15 different drinking water aquifers. Microbial activity and OMP removal were monitored over a period of 80 days, with OMP biodegradation normalized to the biomass content of the different waters. The biodegradability of bulk DOC in each aquifer was analyzed using excitation emission matrix (EEM) to characterize the DOC fractions. We hypothesized that microbial activity and richness as well as availability of biodegradable DOC are important parameters determining OMP biodegradation capacity. With our findings, drinking water companies can more effectively harness the potential of the indigenous microbial community, increasing the viability of nature based solutions to remove OMPs.

3.05.P-We225 Innovative Strategies for Removal of Organochlorine Contaminants: Selection of the Best Tools for Synergistic Abiotic-Biotic Breakdown

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Organochlorine substances (PCBs, chlorinated hydrocarbons, pesticides, e.g.,) are among the most common environmental pollutants. Their diverse properties as chemical stability, low water solubility, and volatility are outlining their environmental fate. Generally, they are toxic, hardly biodegradable, and persistent. The molecular complexity of several substances of this kind, mainly aromatics and alicyclics driving forward the research of the complementary technologies consisting of individual steps, resulting in the effective degradation of contaminant molecule. One of such approaches is nanobiodegradation. First, iron-based nanoparticles dechlorinate the pollutant molecule, and in the second step specialized bacteria cleave the remaining structure. In our study, the iron bionanoparticles (Fe-BNPs) synthesized from the waste-based plant materials were applied and compared with synthetic nZVI nanoparticles. The bacteria were isolated directly from the contaminated matrix by enrichment method using the increasing selection pressure of contaminant. The research was focused on obtaining of the degrading bacterial culture and optimization of the Fe-BNP synthesis so that they are compatible and provide the highest possible efficiency. The results show that plant waste from food industry (peels, seeds, pomace) are a valuable source of bioactive substances which can mediate the formation of Fe-BNPs, yielding high especially when (a) nitrogen atmosphere was maintained during whole process of synthesis, (b) plant extract was combined with low concentration of synthetic reducing agent and (c) pH was adjusted to slightly alkaline values. It was also possible to obtain bacterial culture capable of significant reduction of organochlorines. As the abiotic and biotic processes differs in the oxygen requirements, the further steps are aimed to construct the microcosm setup for sequential nanobiodegradation of organochlorines with potential for latter scale-up to pilot unit, where biodegradation could proceed anoxically and biodegradation in oxic atmosphere. It will relate also to determining of the toxicity of Fe-BNPs to bacterial culture, as well as with searching of the appropriate way for deactivation of Fe-BNPs before biodegradation step. Nanobiodegradation exploits the potential of natural sources as adapted bacteria and gives the added value to the material originally considered waste, thus appears to be an economically and ecologically friendly approach.

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3.05.P-We226 Natural Degradation of Plastic Surfaces in Lake Geneva: Analyzing Depth-Dependent Changes and Microbial Influences

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Plastic degradation in aquatic ecosystems involves abiotic processes, such as photooxidation, and biotic processes driven by biofilm microorganisms producing polymer-degrading enzymes. However, the long-term impact of the natural plastic degradation in freshwater ecosystems remains understudied. This project aims to (1) characterize the biofilm community on low-density polyethylene (LDPE), polyethylene terephthalate (PET), and glass in Lake Geneva, and (2) examine plastic degradation at different depths under reduced light conditions. Samples were incubated for 10 months at 2, 30, and 100 meters, with environmental parameters monitored via the LÉXPLORE platform. Biofilm DNA is extracted for Next-Generation Sequencing (NGS) to identify bacteria, algae, and fungi. Quantitative PCR (qPCR) is performed to investigate the biodegradation potential targeting the alkane hydrolase genes (*alkB*). After biofilm removal, plastic surfaces were analyzed using Fourier Transform Infrared spectroscopy (FTIR) to detect chemical changes, contact angle measurements for hydrophobicity, and Scanning Electron Microscopy (SEM) for surface morphology. The results from NGS indicate that depth and time gradient influences strongly biofilm composition: at 2 meters, a dense biofilm of bacteria, fungi, and algae fluctuates seasonally; at 30 meters, bacteria and fungi dominate with constant growth; and at 100 meters, only a few bacteria persist. Hydrophobicity reduction was most significant at 2 meters, likely due to photooxidation or biotic factors. At 30 meters, PET's hydrophobicity remains stable, while LDPE's surfaces showed a significant decrease in hydrophobicity compared to virgin LDPE. FTIR spectra revealed a strong decrease in CH bonds (methyl group) and the appearance of new carbonyl peaks (1715 cm⁻¹ 1649 cm⁻¹) on LDPE at 30 meters only, suggesting chemical changes on the surface. Noticeable porous cavities are observed with SEM imaging at 30 meters, confirming a possible plastic degradation after 8 months of incubation. Additionally, the abundance of *alkB* increased significantly on LDPE,

whereas it decreased on PET. Heterotrophic bacteria, such as *Sphingomonas*, and saprotrophic fungi, like *Cladosporium*, were identified in biofilms and are promising candidates for plastic degradation. The chemical properties of LDPE, with its aliphatic hydrocarbon structure and lower crystallinity, make it more susceptible to both abiotic and biotic degradation processes than PET.

3.05.P-We227 Biodegradation of Food-Related Plastic Materials by *Penicillium Brevicompactum*
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The pervasive use of plastic polymers, particularly in the food industry, has steadily increased in recent decades, presently posing a problem of urgent environmental concern. These materials, while beneficial for extending the shelf life of various food products, significantly contribute to plastic waste, much of which eventually reaches and contaminates natural environments. Consequently, developing effective and environmental friendly solutions for plastic waste management is of paramount importance.

Herein, we explored a promising approach to mitigating this environmental crisis through the biological degradation of plastic waste. The research focused on the fungus *Penicillium brevicompactum* and its ability to degrade polyethylene (PE), a common type of plastic widely used in the food industry. Two types of PE were selected: high-density polyethylene (HDPE), sourced from a yogurt bottle, and low-density polyethylene (LDPE) obtained from a food storage plastic bag. These materials were chosen due to their prevalence in everyday consumer products and their substantial contribution to plastic pollution. Experimental results revealed that *P. brevicompactum* was capable of colonizing both types of PE, demonstrating its potential as a biological agent for plastic degradation. Although complete degradation of these materials was not achieved, approximately 20% of HDPE and 40% of LDPE were degraded. These findings highlight the potential of fungi in reducing plastic waste through biodegradation.

By harnessing biological mechanisms, such as the action of fungi, more sustainable and eco-friendly practices for managing plastic waste may be developed. The study underscores the importance of continued research in the field of bioremediation and the need for interdisciplinary efforts to combat the growing problem of plastic pollution. Furthermore, results constitute a promising avenue for the application of green chemistry principles in addressing one of the most pressing environmental issues of our time. By leveraging natural processes for the degradation of plastic waste, we can move towards a more sustainable and environmentally responsible future.

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3.06.A Can Biodegradable Polymers Serve as a Safe and Sustainable Solution to Environmental Accumulation of Polymers?

3.06.A.T-01 A Conceptual Model to Overview Microplastic Persistence and Environmental Impact of Biodegradable and Non-Biodegradable Polymers

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Reducing plastic pollution is at the top of the Green Deal agenda. We are looking at plastic applications that, after reduction, redesign, reuse and recycling, still have an input to and thus an impact on the environment. Biodegradable materials can be a solution, but questions remain: Where do they make sense? Do they offer benefit over non-biodegradable plastics? And how do you find the right polymer or material? Full biodegradation leads to a reduction in pollution, which is cited as an advantage in the EU policy framework for bio-based, biodegradable and compostable plastics. The document clarifies that materials and products claiming to be biodegradable should be verified against existing standards and certificates that they are biodegradable and how long it actually takes for them to biodegrade. However, before such verification or even certification can take place, the manufacturer is faced with the question of which material to choose or what biodegradation times are required or should be achieved. We present a novel conceptual model to move forward in these two situations. It combines materials, processes, environmental behavior and effects with the lifetimes of different plastic polymers. We discuss the model which provides a promising baseline for selecting a polymer/material that combines the required functionality with the minimum possible impact, and the reduction of accumulation in the environment.

3.06.A.T-02 Analysis of (Micro)Plastic Biodegradation at Single-Cell Level by Stable Isotope Raman Microspectroscopy

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The ubiquitous use of plastics demands thoughtfulness about their fate in the environment.

Biodegradability is, therefore, a prerequisite for sustainable future applications in cases where plastics cannot be efficiently retrieved, e.g., in agriculture. Here, complete mineralization of the plastic into water, CO₂ and biomass is desired. To this end, the biodegradation process must be systematically studied to reliably classify biodegradable polymers and to design new ones. Conventional methods either analyze the change of polymer properties during degradation (e.g., mass loss or fragmentation) or microbial activity in terms of O₂ consumption or CO₂ production. However, those methods lack a direct relation between plastics and microorganisms, to see which species are responsible for the degradation and to determine the finale fate of plastics. Stable isotope techniques can close this gap by tracing plastics to their ultimate degradation products. Here, we bring forward stable isotope Raman microspectroscopy at the single-cell level to broaden the mechanistic understanding of microbial degradation of (micro)plastics in natural systems. We selected perdeuterated D-poly(lactic acid) (dPLA) as model plastic and traced Deuterium label during incubation experiments into microbial biomass using C-D vibrations (appear in the Raman-silent region of undeuterated biomass, 2050-2300 cm⁻¹). The use of deuterated, instead of ¹³C-labeled polymers, turned out to be a very suitable alternative (i.e., availability, price, and pronounced red-shift in Raman spectra). The 2068 cm⁻¹ C-D band was indicative of strongly deuterated lipids, enabling the detection of metabolic differences during incubation with dPLA (i.e., stronger lipid and weaker protein deuteration) compared to glucose-d₁₂ and D₂O as alternative D sources. Single-cell analysis was the key to detecting phenotypic heterogeneity and classifying cells of the naturally occurring bacterium *Sphingomonas koreensis* in two clusters: one showed a significantly stronger deuteration level than the *Escherichia coli* control, whereas the other was non-labeled. The Deuterium label could even be detected in the strong resonance Raman signal of carotenoids, highlighting the potential for high throughput technologies like imaging and cell sorting. To demonstrate the transferability to environmental samples, experiments were conducted with soil bacteria isolates and D uptake from dPLA into microbial biomass was observed after two weeks.

3.06.A.T-03 Biodegradable Mulch Films in Agricultural Soils: The Effect of Temperature on Biodegradation Dynamics

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Mulch films play a crucial role in modern agriculture to increase crop yields, e.g., by extending growing seasons, controlling weeds, and saving irrigation water. Biodegradable mulch films (BDMFs) are a viable alternative to conventional, environmentally persistent polyethylene films by offering the benefit of biodegrading in soils after use. When assessing biodegradability of BDMFs in field soils, understanding the effect of temperature on biodegradation dynamics is essential. Yet research in this area remains limited. This study investigates the effect of incubation temperature on the biodegradation dynamics of BDMFs containing the biodegradable polyesters poly(butylene adipate-co-terephthalate) (PBAT) and poly(lactic acid) (PLA). To this end, laboratory soil incubations were run at four environmentally relevant temperatures (i.e., 5, 15, 25 and 35°C) in three soils over a period of two years. Biodegradation was followed by quantifying residual PBAT and PLA at five timepoints over the incubation period by Soxhlet extraction of residual polymer coupled to quantitation by proton nuclear magnetic resonance spectroscopy (1H-NMR). This analytical methodology enabled the independent tracking of PBAT and PLA biodegradation. The biodegradation rates and extents of both PBAT and PLA varied between soils but, independent of the soil, were strongly temperature dependent with a general expected increase in biodegradation rates with increasing temperature. Kinetic modeling of the data allowed for the estimation of biodegradation rate constants and activation energies. The 1H-NMR analysis also allowed assessing changes in the monomeric composition of PBAT and revealed an increase in the aromatic diacid (i.e., terephthalate) content with increased biodegradation extent. Modeling efforts, including predictions based on in situ field temperature data, aim to use the laboratory incubation results to predict biodegradation half-lives for the BDMF polymers in the field. The applicability of rate-temperature relationships (e.g., the Arrhenius rate law) will be critically assessed. This work represents a first step toward predicting in situ field biodegradation rates from laboratory incubations conducted at 20-28°C, as specified by the European (EN 17033:2018) and international (ISO 23517:2021) BDMF biodegradability standards.

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3.06.A.T-04 Case Studies that Show the Predictive Modelling of Microplastic Accumulation in the Natural Environment

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The use of plastics inevitably leads to (micro-)plastics entering and accumulating in the natural environment, affecting biodiversity, food security and human health. Biodegradable polymers have emerged as a potential solution to mitigate plastic pollution and the environmental accumulation of polymers, offering stable performance during use while leading to shorter residence times in natural environments. But do we know the effect of these novel polymers on microplastic accumulation in the natural environment?

We developed a comprehensive and universally applicable method to quantify microplastic accumulation in the natural environment. This method includes an integrated biodegradation model that provides the possibility to examine and compare the microplastic formation and accumulation of different polymer types in diverse natural environments. The model uses experimental mineralisation curves of polymers to predict the microplastics accumulation. The model fits the mineralisation curves to a state-space model and therewith generates a mass balance of substances formed during the biodegradation process. This polymer-environment specific mass balance is used to predict the concentrations and residence times of microplastics in the natural environment. The modelling results can be used directly to assess and compare the accumulation of different polymers, and could be used as input for sustainability studies such as a life cycle analysis (LCA). Ultimately, the proposed methodology identifies the required biodegradation behaviour to prevent the microplastic accumulation of specific plastic products in the natural environment. This will facilitate the transition to a system in which products are designed based on their intended functionality and anticipated end-of-life scenario.

For this presentation, the model is applied in different case studies to study the possible benefits of the use of biodegradable polymers as a solution to environmental accumulation of polymers. These case studies are focused on different applications of biodegradable polymers, in different environments. The preliminary results of modelled case studies show that replacing non-biodegradable polymers with biodegradable polymers can be effective in the prevention of environmental microplastic accumulation, even in case of higher littering ratios or the application in agricultural products that are not retrieved from the land.

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3.06.P-We244 Degradation of Biodegradable Plastics

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Decades of extensive production and use of conventional plastics have led to their accumulation in the environment. Bioplastics (bio-based and/or biodegradable) have been promoted as a more sustainable alternative. However, the literature reveals conflicting conclusions regarding their suitability and environmental impact. A primary area of dispute concerns their biodegradability and the conditions necessary for proper degradation. In real-world settings, biodegradable plastics may not degrade as rapidly or efficiently as laboratory tests indicate. Here, we describe three activities that were conducted to address these issues: (1) a systematic literature review to explore the current level of knowledge, (2) a degradation test on consumer products made from biodegradable plastics was conducted based on an identified knowledge gap in the current literature, and (3) development of a novel oil-based method for extracting aged bio-microplastics from compost matrices. The review covered the degradation of biodegradable plastic in waste management environments (e.g., compost, sludge, or landfill) and the open environment (e.g., seawater, freshwater, or soil). The results highlight challenges in comparing and quantitatively analyzing data on plastic degradation due to methodological variations, including differences in testing methods, materials, and quantification strategies. Moreover, several research gaps and limitations exist. Notably, there is a need to intensify research on polyhydroxyalkanoates (PHAs), polybutylene adipate

terephthalate (PBAT), and polybutylene succinate (PBS) to match the level of polylactic acid (PLA) and starch-based plastics. A degradation test was carried out on commercially available biodegradable plastics under simulated industrial composting conditions according to ISO 20200. The results indicated disintegration degrees between 75-100 % for all tested biodegradable plastic products, with five of ten reaching complete disintegration within 90 days. Finally, a method was developed to extract microplastics from these compost samples using oleoextraction. The outcomes of these studies offer valuable insights, particularly in guiding discussions about the prospective role of biodegradable plastics within society. Based on our findings, essential knowledge and data gaps will be highlighted, and suggestions will be provided regarding the direction of future research to assess their role as alternatives to conventional plastics.

3.06.P-We245 Toward Standardization in Microplastics Research: Development of an Extraction Protocol from Compost for ISO

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The current economic model is producing plastics in large quantities, and despite our best efforts to recycle and reuse them, some still end up in the environment. With the potential to have negative impacts on flora and fauna, with the potential to be harmful to humans as well. These plastics age and fragment over time, generating microplastics, which vary in color, shape, and physicochemical properties. These differences in properties between different particles challenge their treatment as only one type of contaminant. Besides that, despite knowing that microplastics are present everywhere, there is still no standard method for their extraction and characterization from environmentally complex matrices. This is why we are in the process to standardize a method for the extraction and characterization of microplastics from compost, with a plan to have it finalized by 2027 or earlier. This standard will allow for reproducible and homogenized results, allowing the comparison of findings between different studies and laboratories. The developed extraction protocol consists of deagglomeration, organic matter removal, and density separation. The organic matter removal is done by Fenton oxidation, and the density separation employs calcium chloride. Not only would it target the extraction of fossil fuel-based non-degradable microplastics, but also fragments from biodegradable polymers. Fragments from biodegradable plastics could be present during the initial stages of the composting process but are not expected to be found in final composting products. To achieve the extraction of microplastics, the extraction protocol, under standardization, does not apply harsh conditions. Current results show that the herein developed method has high recovery efficiencies for PBAT microparticles without affecting non-degradable plastics and has negligible effect on the size of biodegradable ones. For the characterization of the particles, we are using a micro-Raman system that employs a 785 nm laser and a particle finder/analyzer software to automate the process. Currently, an interlaboratory study is ongoing to test and validate the presented extraction protocol. Furthermore, we aim to sample final compost products from industrial composting plants in Austria and Germany, comparing plants serving small and large communities.

3.06.P-We251 The Impact of Fossil and Biobased Microplastics on Human Cells

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Plastic pollution is one of the most significant environmental challenges of the twenty-first century, with microparticles now found in virtually all ecosystems. Humans are exposed to these particles through three primary routes: ingestion, absorption, and inhalation, and the potential effects largely depend on the composition, shape, and size of the particles. While most research on micro- and nanoplastics focuses on spherical or rod-shaped particles, with polystyrene (PS) being the most commonly studied polymer, the most widely produced plastics are polypropylene (PP) and low-density polyethylene (LDPE). Additionally, there has been a rise in the production of biobased polymers, which degrade faster than traditional fossil-based plastics in an effort to mitigate the harmful effects of conventional plastics. However, the impacts of these newer materials remain largely unknown. To better understand the effects of micro- and nanoparticles on human health, five types of plastic three from fossil sources (LDPE, PS, and PP) and two from biological sources (PLA and PHB) were mechanically degraded and separated by size using stainless steel mesh filtration and the fraction below 1.6 µm was chosen. Human cell lines from the liver (HepG2) and intestine (HCT116) were used as biological models and exposed to eight different concentrations of each plastic type and size range, starting from 1.28 µg/L to 100 mg/L. Cellular viability was assessed at three time points (24, 48, and 72 hours) using the MTT assay. In general, HCT116 cells showed greater sensitivity to the plastics than

HepG2 cells, with toxicity being similar between fossil-based micro- and nanoplastics, but biobased present a higher impact than biobased microplastics. Our findings highlight the importance of further investigating the impact of novel biobased and biodegradable polymers on human health.

3.06.B Can Biodegradable Polymers Serve as a Safe and Sustainable Solution to Environmental Accumulation of Polymers?

3.06.B.T-01 Polymer Biodegradability 2.0: A Holistic View on Polymer Biodegradation in Natural and Engineered Environments for a Reliable Product Development

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Biodegradable polymers are an important part of the solution toolbox to achieve circularity (biological cycle). Therefore reliable biodegradable polymers need to achieve not only an appropriate performance level in applications e.g., home and personal care to fulfill their function during the use phase but also on a biodegradation performance level after use. The biodegradation performance is tailored to the application and the receiving environment of the polymer product after use, which can be both engineered systems (e.g., wastewater treatment plants) and natural systems (e.g., soils, freshwater, or marine environments). This presentation addresses key aspects of polymer biodegradability and biodegradation in both natural and engineered systems with the goal to advance a more holistic view on the topic and, thereby, provide guidance for all stakeholders working on developing, testing, and regulating biodegradable polymers. These aspects include definitions of biodegradability and biodegradation, elucidating polymer- and environmental factors that control the biodegradation process, a discussion of the analytical chemistry of polymer biodegradation, polymer biodegradability testing and certification, the contribution of digital tools like predictive modelling, as well as a brief overview of research needs. In addition, Biodegradability 2.0 includes a joint approach of academia, industry, associations and political decision makers to enable the development of new high performing reliable biodegradable polymers in respective applications e.g., for home and personal in the future.

3.06.B.T-02 Relationships Between Structure and Soil Biodegradability for Synthetic Polyesters: Tracking Carbon using Stable-Isotope Labelling

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Replacing conventional plastics in some applications with those designed to be biodegradable is one key strategy to reduce well-documented accumulation of plastic pollutants in the open environment. The application of biodegradable polymers necessitates an in-depth understanding of the controlling factors on their biodegradability under environmental conditions.

Here, our goal is to understand structure-biodegradability relationships for different, novel, synthetic polyesters. To this end, we synthesize stable carbon isotope (^{13}C)-labelled polyesters, and investigate their soil biodegradability in controlled laboratory incubations. We utilize isotope-selective analytics to track polyester carbon to CO_2 and to close mass balances on polyester carbon during aerobic biodegradation. By quantifying residual polyesters in the soil, we can infer their assimilation into microbial biomass, comprehensively understanding their microbial utilization.

In our studies, we have looked into different polyester classes of emerging interest, with varying structures. One class is aliphatic-aromatic copolyesters based on polybutylene adipate-co-terephthalate (PBAT), with changes to the aliphatic monomer identity (sebacate (Se) vs. adipate (A)), and changes to the ratio of aliphatic to aromatic monomer units. Another type is long-chain aliphatic polyesters, such as polyester-2.18 (PE-2.18), which have polyethylene-like solid-state structures and tunable properties based on changes in the chain length of the long-chain aliphatic monomers.

Overall, we find strong dependence of soil biodegradation of synthetic polyesters, including mineralization and assimilation into microbial biomass, on their monomer structures. These relationships, together with related structure-property relationships for key materials properties, are key to inform the design of biodegradable polymers for environmental applications.

3.06.B.T-03 Review of Experimental Approaches and Analytical Methods for Biodegradability and Persistence Assessment of Water-Soluble Polymers

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Water-soluble polymers (WSP) are important and diverse classes of macromolecules that are used in a

wide variety of applications, including home and personal care and agricultural formulations. There is a need to better understand the biodegradation, and broader fate, of WSPs in engineered and natural systems that receive these polymers after their use, and to develop robust methods to evaluate this process. Polymers of high molecular weight are not directly available for uptake into microbial cells, preventing these polymers from direct intracellular metabolic processing. These polymers therefore often require initial extracellular breakdown into smaller chemicals. Due to diverse chemical structures and molecular weights of WSPs, a number of physicochemical properties may affect their biodegradation. The interpretation of biodegradation data can be challenging due to the complex composition of WSPs. Finally, complete biodegradation requires many transformation steps which individually may be difficult to detect. Taken together, these WSP-specific considerations challenge the applicability of current biodegradability and persistence assessment frameworks developed for low molecular weight organic chemicals. There is a need to critically assess the transferability of these frameworks to WSPs and, if needed, propose scientifically-founded yet practical frameworks for biodegradability assessment of WSPs. To address this need, we conducted a comprehensive review of available methods and policy frameworks for assessing the biodegradation and environmental persistence of WSPs. The aims of this work were to (1) investigate the applicability of current biodegradability and persistence frameworks to WSP; (2) review available standard and experimental methods, addressing their applicability to the assessment of WSP biodegradation; (3) develop recommendations for future research and advancements in these areas. Scientific studies on this emerging topic remain scarce, highlighting the need for further research. Various promising experimental approaches for assessing WSP biodegradation have been identified and we considered these in the context of applicable regulatory frameworks and possible shortcomings identified therein. Existing and emerging policies suggest that assessing WSP biodegradation will be increasingly required.

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3.06.B.T-04 Photochemical Chain Scissions Enhance Polyethylene Glycol Biodegradability: From Probabilistic Modelling to Experimental Demonstration

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Polyethylene glycols (PEGs), a prominent group of water-soluble polymers (WSPs), are extensively used across multiple sectors, leading to potential environmental release. This study investigates the interaction of PEGs with photochemically generated hydroxyl radicals ($\text{OH}\cdot$), a highly reactive oxidant found in natural environments, and examines how the resulting molecular weight (MW) reduction influences PEG biodegradability in soil and sediment. A probabilistic kinetic model was developed to simulate the production of low-MW PEG fragments through $\text{OH}\cdot$ -driven chain scissions, revealing that MW drops significantly with only a few scission events per original chain. To validate the model, experimental reactions were conducted using ^{13}C -labeled PEGs (initial average MW = 6200 Da) exposed to $\text{OH}\cdot$, which showed a strong decrease in MW, closely matching the model predictions. Subsequent 150-day soil and sediment incubations of both untreated and $\text{OH}\cdot$ -treated PEGs revealed that lower MWs, achieved via $\text{OH}\cdot$ reaction, led to faster and more extensive mineralization to $^{13}\text{CO}_2$. This research highlights the importance of considering full MW distributions and their transformation through biotic and abiotic chain scissions, as illustrated by PEGs reaction with photochemically generated $\text{OH}\cdot$, for a comprehensive understanding of WSP biodegradability in various environmental settings.

3.06.P-We233 Developing a High-Throughput Biodegradation Test to Investigate the Influence of Microbial Inocula on Biodegradation Outcome in Polymers

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Polymers are key components in many homecare products, from pharmaceuticals and cosmetics to detergents and sun cream. However, the range of methods available to determine the fate of these polymers in the environment after their disposal is limited, with most being designed for smaller, low-molecular weight compounds. Additionally, existing biodegradation tests are resource-intensive and low-throughput, often only providing a few replicates over 28 days. To address this research gap, we have developed a quick, simple and high-throughput biodegradation test method for water-soluble polymers. This utilises a cresol red-based gel in a 96-well plate setup to measure CO_2 released by microbial inocula as the polymer is degraded. The plates are inoculated with an environmentally relevant microbial inoculum with the polymer and then clamped to a second plate with the detection gel to form a gas-tight

environment. The gel's colour change can be measured with a spectrophotometer and used to calculate a mass of CO₂ produced. With CO₂ as the analytical endpoint and a 96-well plate format, hundreds of replicates for a given polymer can be produced in the same time that a standard test could produce just a few. The method is being tested with the polymers pullulan and dextran against a range of inocula including activated sludge from wastewater treatment. Our primary aim is to determine if the method can detect clear differences in biodegradation outcome of a given polymer when exposed to differing concentrations of inoculum. This would provide validation of the method's effectiveness. We further hypothesise that significant differences will be found in the biodegradation of a given polymer depending on the inoculum type it is exposed to such as activated sludge versus river water communities. This new high-throughput biodegradation test could increase our capacity to rapidly assess the wide range of new polymers currently in development. Therefore, paving the way for more biodegradable products and better protection for the environment post-disposal as well as allowing us to study the factors affecting biodegradability in greater detail.

3.06.P-We235 Polyquaternium Polymers in the Aquatic Environment: Analytical Method Development and First Occurrence Data

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Polyquaternium (PQ) compounds are a highly variable sub-class of water-soluble polymers (WSPs) that share a quaternary ammonium substructure as their common structural element. Due to their high production volumes, wide-spread use, first evidence of ecotoxicity, and assumed persistence in the environment information on their occurrence and environmental concentrations is essential to assess the risk these chemicals may pose and if regulatory action is required. However, typical trace-analytical methods cannot be applied for PQ-compounds since the total intensity of the polymer in electrospray-ionization mass spectrometry is spread out over countless signals. These originate from the polymer's polydispersity, charge states, and adduct formation and are nearly indistinguishable from background noise even in high concentrations and pure standards. To enable the detection of four selected PQs (PQ-2, PQ-6, PQ-7, and PQ-10) in environmental matrices in-source fragmentation was provoked, converting polymers of different chain lengths, charge states, and adducts to diagnostic fragments of higher intensity than any individual polymer signal.

The developed method was used to screen for these four PQs in 15 surface water samples, which led to the widespread detection of PQ-2 (13/15 samples) after direct injection and the detection of PQ-7 and PQ-10 in samples with a strong urban impact after freeze drying (20-fold enrichment). PQs were shown to interact strongly with humic acids, hampering their analysis and combined with the lack of well-characterized standards this prevents accurate quantification with the current iteration of the method. Nevertheless, PQ-concentrations were estimated in the tens to hundreds of µg/L for many of the investigated samples and reached up to a 1 mg/L for the most contaminated sample. These estimated concentrations seem remarkably high and thus require confirmation with orthogonal methods and, if confirmed, a regulatory follow-up.

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3.06.P-We252 Screening of the Environmental Safety of a Novel Biomaterial for Use as Alternative for Plastics

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Bioplastics are products with enhanced biodegradability and/or of biobased source whose growing production has the potential to replace conventional petroleum-based plastics. Natural biopolymers such as chitosan and cellulose are the most abundant on the planet and can be used for bioplastic production. Here, a newly developed chitosan-methylcellulose bioplastic was investigated for its environmental safety in terms of its disposal after use in terrestrial environments as compost. For this, ecotoxicological tests using three higher plant species exposed to different concentrations of the tested bioplastic were run. Bioplastics were mixed in standard soil at 0.1, 1, and 10% (w/w) concentrations, and parameters of seed emergence, root growth and shoot growth were measured after 3 and 6 days after seed incubation in dark at 25±1°C. The value for effective concentration to observe 50% of effect (EC₅₀) was also estimated. There was a significant decrease in seed germination in seeds exposed to the 10% bioplastic treatment (avg. 8.3%) when compared to the control (avg. 98.89%). Previous studies have shown that plant seeds

exposed to conventional plastic particles can have delayed seed emergence, but this could not be verified here as experiment was terminated at day 6. We hypothesize that delays in seed emergence might be related to reduced water availability caused by the bioplastic hygroscopic nature, and this is being currently tested. Root growth and shoot growth followed a similar pattern as seed emergence, with many zero values for root/shoot length at the 10% bioplastics treatment. EC50 values varied among species, and the dose-response curves highlight the datapoint gaps between treatments of 1%-10% bioplastics which calls for further experiments with a refined range of concentrations. In summary, the tested chitosan-methylcellulose bioplastic material showed no significant effects on seed emergence, root length, or shoot length in tested plant species at concentrations up to 1% bioplastic in soil (w/w). There appears to be a transient effect at a 10% concentration that can be related to changes in water availability caused by the bioplastic addition, and further tests are underway to confirm this. This study adds to the potential for using biobased materials to replace persistent, toxic plastics and for safely disposing the tested bioplastic in terrestrial environments as compost.

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3.06.P Can Biodegradable Polymers Serve as a Safe and Sustainable Solution to Environmental Accumulation of Polymers?

3.06.P-We228 Biodegradation of Water-Soluble Polymers – Reviewing In-Silico Methods

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Polymers have been in long-term, broad use by various industries. Evaluating their environmental impact is essential, especially within the framework of the European Green Deal and the Chemicals Strategy for Sustainability. While polymers have been exempt from mandatory environmental fate scrutiny under REACH (EC 1107/2009), this exemption is going to be revised. A critical component of this evaluation is the assessment of degradability and persistence of functional polymers, such as those used in cosmetic formulations.

Biodegradation modelling for polymers can employ most notably Quantitative Structure-Activity Relationship (QSAR) models or molecular modelling simulations representing statistical and deterministic approaches. However, common QSAR models for biodegradability, such as BIOWIN in EPI Suite, cannot be extended to polymers due to their stochastic structures. While QSAR tools like Polymer Genome exist for deriving polymer properties, biodegradability prediction has not yet been implemented in any of them. Molecular modelling, although computationally demanding, is capable of estimating polymer properties but still needs to be adapted for biodegradability predictions.

This poster explores the current landscape of in silico methods for assessing polymer degradation. It argues that teaching polymer-specific models to predict biodegradability is more feasible than modifying existing biodegradability tools (including pathway prediction systems) to handle polymers. In an underlying database, biodegradability would be considered as a property. Polymer databases, however, have to capture their stochastic nature. The need for advancing these methodologies is highlighted, with suggestions for refining in silico approaches to improve the understanding of polymer environmental fate.

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3.06.P-We229 Applicability and Improvements of OECD (Bio)degradation Testing for Water-Soluble Polymers

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Biodegradation screening testing and more complex biodegradation simulation studies are requested under regulatory frameworks such as REACH for persistency assessment of low molecular weight (small) molecules. While polymers and polymeric substances were initially exempted from REACH, this will change in the near future, and implementation of polymer-specific modifications of the respective ordinances and guidance are necessary. In this context, the question arises whether standard test guidelines used for the determination of small molecules degradability are appropriate for polymers. These guidelines

might need modifications to obtain meaningful data on the biodegradation of polymers in environmental compartments. Possible modifications are not limited to the test setup and technical aspects, but are concerned more with degradation endpoints, which will have to be different compared to small molecules (e.g., changes of the molecular weight distribution, definition of primary degradation). While there is much attention paid to degradation of plastics due to the microplastic restriction, so far studies on water-soluble polymers such as e.g., PEGs or PVOHs are largely missing, especially with regard to higher-tier biodegradation simulation studies.

The CEFIC LRI ECO64 project makes a start to close these gaps. In a first step ¹⁴C-radioactive labelled water-soluble polymers will be synthesized, e.g., PVOH and/or CMC. With these compounds available, their biodegradation in the four most important biodegradation simulation tests (soil, sediment, surface water and WWTP-simulation) should be investigated. In parallel these compounds will also be applied to standard screening tests for comparison reasons. In addition to the aforementioned substances, also a radiolabelled biocidal polymer will be tested in soil, water/sediment and WWTP simulation tests. Using the specificity of the radioactive labelling, endpoints possibly relevant for polymer biodegradation beyond the parameter mineralisation will be investigated. Based on the results of the biodegradation simulation and screening tests, recommendations for polymer testing and also appropriate reference substances should be derived.

3.06.P-We230 Scientific and Regulatory Challenges for Assessing the Biodegradability Testing of Fragrance Encapsulates

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The rising demand for sustainable solutions in the fragrance industry, coupled with the EU's restriction on synthetic polymer microparticles, has driven the development of biodegradable fragrance encapsulates that meet OECD tests, such as OECD 301B or OECD 301F. Internal and external studies confirm these encapsulates achieve >60% biodegradation, ensuring their global applicability.

To tackle biodegradation testing challenges, we have applied standard test conditions while enhancing testing volume and duration. However, we still investigate issues related to low inoculum concentrations in typical settings (usually 30 mg/L) compared to the significantly higher suspended solids concentrations in wastewater treatment plants. We are exploring inoculum from sources like activated sludge or soil and other modifications to improve biodegradability assessments. Additionally, we are testing raw materials for their biodegradability to increase the likelihood that the final polymer is also biodegradable.

Understanding the biodegradation pathways and kinetics of polymers is also a challenge. Due to their non-soluble nature, analytical methods such as LC-HRMS, used for identifying metabolites in the case of low molecular weight chemicals, are of limited use for solid polymers. Furthermore, physicochemical analyses, e.g., partition coefficients, pose significant challenges due to the intricate nature of polymer compared to traditional fragrance assessments. To address these issues, we investigate employing techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA), Pyrolysis-GC-MS, electronic microscopy, and Total Organic Carbon (TOC) measurements. However, the isolation of the intermediates from organic matter remains a difficult task, as does the interpretation of the analytical result in case of analysis of samples in presence of the inoculum as matrix. Another regulatory hurdle is the varying regulatory standards across regions, for example, the necessity to conduct biodegradability tests in e.g., Japan (OECD 301C or OECD 301F), where test performed outside the country are not recognized.

In conclusion, our findings not only advance the understanding of biodegradable fragrance encapsulates but also help overcome some regulatory and experimental challenges in the fragrance industry. By ensuring compliance with inter-national standards, we pave the way for more sustainable practices that align with global environmental goals.

3.06.P-We231 Water-Soluble Polymers Going Down the Drain: Assessing Biodegradation by Wastewater and Freshwater Microbiomes and Adsorption to Activated Sludge

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Water-soluble polymers (WSPs) are essential components in many consumer products, such as household cleaners and personal care items. After use, WSPs used in these products are often discharged into wastewater systems, where various processes such as biodegradation and adsorption to sewage sludge determine to what extent WSPs are removed from wastewater or released into the natural aquatic environment. For a better understanding and prediction of the fate of WSPs during wastewater treatment processes and in natural aquatic environments, it is important to study the biodegradation of WSPs by microbiomes in these systems and the adsorption of WSPs to activated sludge and how these processes are

affected by the properties of a WSP (e.g., charge state). In this study we conducted biodegradation and adsorption experiments with three selected WSPs with varying charge states (i.e., polyethylene glycol (PEG), poly(lysine) (PLL), charged carboxymethyl-dextran (CM-DEX)). Biodegradation experiments were conducted using freshly collected activated sludge from a municipal WWTP, as well as river water sampled upstream and downstream of the WWTP, following a protocol adapted from the OECD 301F guidelines. Sludge adsorption experiments were performed using fluorescently labeled (FITC-tagged) WSPs, with adsorption monitored via fluorescence measurements using a microplate reader. All polymers exhibited promising biodegradation dynamics by wastewater microbiomes. PEG and PLL showed distinct lag phases over several days, followed by rapid biodegradation before reaching a plateau. In contrast, CM-dextran showed an immediate onset in biodegradation but afterwards displayed a more gradual biodegradation process. WSPs were also biodegraded in river water, but with high inter-experimental and inter-replicate variabilities. No significant differences were observed between upstream and downstream river inocula. Adsorption experiments revealed concentration-dependent results for PEG supporting the usefulness of the chosen assay. For the other tested WSPs, the tested assay came with challenges and has to be optimized. Together, this study presents insights into how to conduct environmental fate experiments of WSPs going down the drain at their end of life.

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3.06.P-We232 Enzymatic Hydrolyzability of Biobased and Biodegradable Polyesters: Towards Structure-Reactivity Relationships

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Biodegradable polymers can play an important role in achieving a circular plastic economy, particularly in cases where collecting and recycling used plastic is challenging (e.g., soil-biodegradable mulch films, compostable biowaste bags). While biodegradable, many of these polymers are still (partly) fossil-based. Transitioning to fully bio-based polymers could reduce greenhouse gas emissions and dependency on fossil resources.

Poly(butylene adipate-co-terephthalate) (PBAT), a widely used biodegradable aromatic-aliphatic copolyester, is typically fossil-based. Replacing PBAT's fossil-derived monomers (terephthalic acid and adipic acid) with bio-based alternatives such as the promising 2,5-furandicarboxylic acid is therefore desirable.

In this study, fully bio-based aromatic-aliphatic copolyesters were synthesized using combinations of bio-based diols, aliphatic diacids, and an aromatic diacid (2,5-furandicarboxylic acid). By varying the ratio of aliphatic-to-aromatic diacid for each combination, a total of 28 unique copolyesters were prepared. Thin films of these copolyesters were then produced via solvent casting.

To evaluate biodegradability, the enzymatic hydrolysis of these copolyester films was assessed using two microbial carboxylesterases: *Rhizopus oryzae* Lipase (RoL) and *Humicola insolens* Cutinase (HiC). Hydrolysis was monitored by measuring dissolved organic carbon, while ¹H-NMR was used to identify hydrolysis products and characterize the residual polyester structures.

The results highlighted three key findings. First, enzymatic hydrolysis strongly depended on the ratio of aliphatic-to-aromatic diacid. Higher aromatic diacid fractions led to reduced hydrolytic activity for both enzymes, emphasizing the importance of this ratio in determining biodegradability. Second, HiC exhibited significantly higher hydrolytic activity compared to RoL, efficiently hydrolyzing most copolyesters within five days. In contrast, RoL showed limited activity, particularly for copolyesters with aromatic diacid fractions of 50% or more. Third, replacing (fossil-based) terephthalic acid with 2,5-furandicarboxylic acid markedly improved hydrolyzability, suggesting enhanced biodegradability with this bio-based aromatic component.

In conclusion, this study establishes clear relationships between copolyester molecular structure and enzymatic hydrolyzability, providing a framework for designing fully bio-based, biodegradable aromatic-aliphatic copolyesters.

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3.06.P-We233 Developing a High-Throughput Biodegradation Test to Investigate the Influence of Microbial Inocula on Biodegradation Outcome in Polymers

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Polymers are key components in many homecare products, from pharmaceuticals and cosmetics to detergents and sun cream. However, the range of methods available to determine the fate of these polymers in the environment after their disposal is limited, with most being designed for smaller, low-molecular weight compounds. Additionally, existing biodegradation tests are resource-intensive and low-throughput, often only providing a few replicates over 28 days. To address this research gap, we have developed a quick, simple and high-throughput biodegradation test method for water-soluble polymers. This utilises a cresol red-based gel in a 96-well plate setup to measure CO₂ released by microbial inocula as the polymer is degraded. The plates are inoculated with an environmentally relevant microbial inoculum with the polymer and then clamped to a second plate with the detection gel to form a gas-tight environment. The gel's colour change can be measured with a spectrophotometer and used to calculate a mass of CO₂ produced. With CO₂ as the analytical endpoint and a 96-well plate format, hundreds of replicates for a given polymer can be produced in the same time that a standard test could produce just a few. The method is being tested with the polymers pullulan and dextran against a range of inocula including activated sludge from wastewater treatment. Our primary aim is to determine if the method can detect clear differences in biodegradation outcome of a given polymer when exposed to differing concentrations of inoculum. This would provide validation of the method's effectiveness. We further hypothesise that significant differences will be found in the biodegradation of a given polymer depending on the inoculum type it is exposed to such as activated sludge versus river water communities. This new high-throughput biodegradation test could increase our capacity to rapidly assess the wide range of new polymers currently in development. Therefore, paving the way for more biodegradable products and better protection for the environment post-disposal as well as allowing us to study the factors affecting biodegradability in greater detail.

3.06.P-We234 Assessing the Biodegradation of Polymers Under Realistic Environmental Conditions

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As focus on sustainable-by-design biodegradable polymers increases, research is needed to evaluate methods to accurately assess polymer biodegradation. Limited research has been conducted on methods to access the biodegradation of polymers under realistic environmental conditions (i.e., simulating real world test chemical concentrations and inoculum levels). This poster will discuss recent research on radiolabeled polysaccharides ([¹⁴C-Carboxyl] Dextran, [³H] Carboxymethyl Dextran, and [³H] Carboxymethyl Cellulose) under laboratory simulated realistic environmental conditions using activated sludge as the receiving compartment. Primary biodegradation, ultimate biodegradation, and transformation intermediates (formed during biodegradation) were quantified using liquid scintillation counting, and as applicable, thin layer chromatography (Rad-TLC) or gel permeation chromatography (Rad-GPC) coupled with a MicroBeta2® microplate counter. Additionally, variability in activated sludge from multiple wastewater treatment plants (WWTPs) as well as river water as the receiving compartment will be explored.

3.06.P-We235 Polyquaternium Polymers in the Aquatic Environment: Analytical Method Development and First Occurrence Data

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Polyquaternium (PQ) compounds are a highly variable sub-class of water-soluble polymers (WSPs) that share a quaternary ammonium substructure as their common structural element. Due to their high production volumes, wide-spread use, first evidence of ecotoxicity, and assumed persistence in the environment information on their occurrence and environmental concentrations is essential to assess the risk these chemicals may pose and if regulatory action is required. However, typical trace-analytical methods cannot be applied for PQ-compounds since the total intensity of the polymer in electrospray-ionization mass spectrometry is spread out over countless signals. These originate from the polymer's polydispersity, charge states, and adduct formation and are nearly indistinguishable from background noise even in high concentrations and pure standards. To enable the detection of four selected PQs (PQ-2, PQ-6, PQ-7, and PQ-10) in environmental matrices in-source fragmentation was provoked, converting polymers of different chain lengths, charge states, and adducts to diagnostic fragments of higher intensity than any individual polymer signal.

The developed method was used to screen for these four PQs in 15 surface water samples, which led to the

widespread detection of PQ-2 (13/15 samples) after direct injection and the detection of PQ-7 and PQ-10 in samples with a strong urban impact after freeze drying (20-fold enrichment). PQs were shown to interact strongly with humic acids, hampering their analysis and combined with the lack of well-characterized standards this prevents accurate quantification with the current iteration of the method. Nevertheless, PQ-concentrations were estimated in the tens to hundreds of µg/L for many of the investigated samples and reached up to a 1 mg/L for the most contaminated sample. These estimated concentrations seem remarkably high and thus require confirmation with orthogonal methods and, if confirmed, a regulatory follow-up.

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3.06.P-We236 Eco-Friendly or Ecologically Disruptive? Investigating Biodegradable Wipe Breakdown and Their Effects on Freshwater Microbial Communities

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Wet wipes are a prominent source of plastic pollution, entering freshwater environments via wastewater treatment systems, where they may be discharged through raw sewage, or shed microfibrils that can bypass stringent treatment procedures. Plastic microfibrils have been observed to persist in the environment and elicit negative ecological and ecotoxicological impacts. Therefore, biodegradable wipe alternatives are increasingly sought after and assumed to have little environmental impact. However, the degradation behaviour of wipe products in natural systems, or their ecological impacts, are relatively unknown beyond controlled condition investigations. Therefore, it is not currently possible to guarantee their sustainability or environmental safety. This study aims to assess how different plastic and biodegradable wipe products behave aquatic environments. This was achieved by carrying out a field experiment in a natural river system, placing wipe products in the environment for a period of 8 weeks. Every 2 weeks, wipe degradation was analysed utilising scanning electron microscopy and infrared spectroscopy. At experiment termination, eDNA samples of wipe biofilms were collected, and 16S sequencing performed to assess the potential changes to microbial communities that colonise and decompose the materials. The results of this field experiment will then be compared to controlled condition degradation experiments, such as the ISO 4892-2 method, utilising enhanced temperatures and irradiance to represent accelerated ageing under solar radiation. This will allow the separation and comparison of physical and biological factors during degradation. We hypothesise that biodegradable alternatives will show considerable degradation compared to their plastic counterparts in both field and laboratory experiments. Their microbial communities may differ, with expected greater abundance and diversity on biodegradable materials. However, the addition of chemical additives to commercial wipe products may influence microbial community composition and diversity, which may have implications for their degradation and potential ecological impact. Gaining a deeper understanding of how biodegradable wipe alternatives behave in natural systems, their residency, and their potential ecological impacts, is paramount to ensure their adoption is a more sustainable and less environmentally impactful decision than their plastic counterparts.

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3.06.P-We237 Degradation of Bioplastics and Related Compounds in a Conventional WWTP Process by Activated Sludge

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Bioplastics have been used as a promising solution to fossil-based plastic contamination. These, produced from renewable sources such as starch or corn, that can be biodegraded under different conditions, it is not clear how they are degraded in waste water treatment plants. In addition, it is necessary to evaluate the possible leaching of plastic additives added in bioplastics, used to provide them with specific resistance or malleability, during the depuration process by microorganisms present in the conventional activated sludge (CAS) as well as their subsequent possible elimination or transformation. In this context, we evaluated the elimination of different (bio)microplastics, possible leaching of plastic additives and related compounds and their further elimination facilitated by CAS organisms at laboratory scale. Two

commercial garbage bags made of polylactic acid (PLA), as an example of biopolymer, was fragmented to microsize and exposed to CAS. The main results showed the leaching of different plastic additives and among the compounds tentatively identified by Compound Discoverer, 22 were confirmed at level 1 of confidence through standards comparison. Of these 22 compounds, some of them increased their presence along the experiment indicating the leaching and no degradation. This is the case of abietic acid, tris(2-butoxyethyl) phosphate, linoleic acid and Uvinul® 3049 from PLA bag. Finally, comparing these results with fossil-based bags made of PE, these showed that the plasticizers tris(2-butoxyethyl)phosphate and diethyl phthalate increased their presence during the first week of the experiment due to their leaching while they suffered a decrease during the second one because of their elimination or transformation by microorganisms. In the case of the elimination of bioplastics by CAS, a decrease of 98% was observed for biobags reaching the maximum elimination percentage followed by PLA pellets with a 87%. This elimination facilitated by CAS microorganisms that use them as a source of energy is more efficient in the case of biobag because of the surface area to volume ratio that facilitates the microbial accessibility as well as for the higher presence of plastic additives related to degradation that facilitates this process. This process needs to be further evaluated to assure that no-toxic compounds or degradation compounds are released to the media.

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3.06.P-We238 Relating Biotic Degradation to Polymer Characteristics to Better Predict the Fate of Biodegradable Plastics in the Environment

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 Polylactic acid (PLA) and bioplastics alike have a designed degradability to avoid the environmental buildup petro-plastics have created. Yet, to achieve sustainable implementation of these polymer technologies, it is important to characterize the designed biotic-degradation on plastics that have been weathered abiotically, which will occur in their use and supposing their improper disposal in the environment. This work seeks to establish methods that explore biodegradability on a fundamental level considering that biotic degradation is a multi-step, synergist process. Therefore, we explored the interplay between enzyme hydrolysis and biofilm formation on photo-aged PLA. For PLA, biotic degradation was primarily driven by photo-induced reduction in molecular weight that allowed for greater enzyme hydrolysis. Biofilm formation of model organism *Shewanella oneidensis* was uniformly enhanced with enzymatic hydrolysis, though the characteristics of the biofilm, as quantified by characterizing the extracellular polymeric substance of the biofilms, show the bacteria were more impacted by the extent of photodegradation. Ongoing work to characterize the influence of other plastic characteristics, such as additives, is being pursued. Overall, this work reveals the importance of characterizing the biodegradation with a more complex system. Additionally, our work emphasizes the importance of exploring the interplay between abiotic weathering and biodegradability, which ultimately can inform optimization of production and disposal.

3.06.P-We239 Evaluating Bioplastic Degradation and Fragmentation Using Rainfall Simulation and UV Ageing

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The accumulation of polymers in the environment due to improper disposal has led to growing concern over their pollution and degradation of ecosystems. Biodegradable polymers are frequently marketed to consumers a sustainable alternative to fossil fuel-based polymers. This has led to a change in consumer behaviour and, often, a misunderstanding that biodegradable polymers are less harmful for the environment than conventional polymers when improperly disposed of. Recent years have seen advancements in biodegradable polymer applications along with their production and use, and it is the expectation that this trend will continue. Although biopolymers are often regarded as a sustainable alternative, the environmental impacts resulting from the improper disposal of biopolymers is an area of research that still contains a significant knowledge gap. Within the EU, Ireland generates the highest amount of plastic packaging waste. The expected growth in biopolymer production along with a gap in the understanding of biopolymer degradation, bundled with Ireland's high plastic waste generation and abundance of rain serve as the foundation of this research. This research aims to evaluate the degradation of degradable biopolymers and nondegradable conventional polymers in a controlled laboratory environment, simulating maritime conditions to gain a better understanding of their degradation in an open

environment due to mismanagement or improper disposal. Rainfall simulators replicate naturally occurring rainfall events and provide quantifiable data. In this research, biodegradable and nonbiodegradable polymers undergo rainfall simulation events for twelve weeks. Post rainfall simulation, the polymers are placed in a cold room and exposed to UV ageing lights under controlled conditions with the aim to analyse polymer degradation over time. Rainfall simulation runoff is collected and will be analysed for microplastic persistence to glean insight into possible impacts to environmental receptors such as soil and waterways. Following the rainfall simulation, mechanical testing in the form of tensile strength tests, and imaging through scanning electron microscopy will be performed to identify and analyse any degradation. To our knowledge, this is the first study of its kind using rainfall simulation to evaluate biopolymer degradation and this research has the potential to offer a novel method for evaluating polymer degradation in a simulated oceanic climate environment.

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3.06.P-We240 Complete Quantification of Products Generated by PBAT and LDPE during Abiotic Degradation Using a Carbon Balance Protocol

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Plastic pollution is everywhere and might threaten life and ecosystem on a global scale as part of the novel entities group[4,5]. Yet, it is still challenging to assess the impact of any plastic object discharged in the environment, partly because of the diversity of the degradation products (microplastics, nanoplastics, soluble and volatile products) and the difficulty to quantify them. Although the use of carbon as a marker for degradation has already been done for some products, we propose here a novel method for the simultaneous quantification of all degradation products generated by a plastic under abiotic degradation: the carbon mass balance protocol.

We choose to compare the abiotic degradation of two types of industrial plastic granules with different chemical composition but similar use: LDPE that is one of the main plastic used worldwide, and PBAT, used as a biodegradable replacement in some applications. Samples were subjected to a variable UV exposure time (500-3500/4000h) before being stirred in water for 3 days. We separated and analyzed the resulting fragments by weighting and elemental analysis to get their carbon mass. The nanoplastics and solubles were subjected to TOC measurement.

Thanks to this protocol, we made a complete quantitative degradation assessment for both plastics and were able to compare the abiotic degradation of LDPE and PBAT granules, showing great difference in their behavior: the LDPE generated a lot more carbon products and was the only one to significantly produce MPs (~7% of the initial carbon mass at 4000h). Unexpectedly, relatively large amounts of volatile products are also generated for each product, highlighting the importance of taking in consideration all degradation products.

Coupling those results with SEM observations of the surface cracking for both plastic gives insights to assess environmental persistence of plastics.

3.06.P-We241 Biodegradation Rates of Polylactic Acid Microplastics in Aquatic Environment: Role of Particle Size and Environmental Aging

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The increasing adoption of biodegradable plastics as alternatives to conventional plastics aims to reduce environmental pollution. While these materials degrade more rapidly than traditional plastics, their accelerated breakdown may lead to a higher production of microplastics (MPs). Once released into aquatic environments, these MPs undergo aging processes such as physical abrasion, chemical oxidation, weathering, and UV irradiation, which modify their physicochemical properties. These changes, particularly in surface area and functional groups, influence the rate of microbial degradation, as degradation predominantly occurs on particle surfaces. This study investigated the biodegradation of polylactic acid (PLA) microplastics (MPs) of different sizes (5, 20, and 150 μ m) and aging states (unaged, thermal aged, and UV/H₂O₂ accelerated aged) over a 90-day period using activated sludge. The results demonstrated that smaller particle sizes exhibited faster biodegradation rates, with the rate constant (k, day⁻¹) increasing 0.0006 to 0.0027, highlighting the critical role of surface area in the biodegradation of MPs. Additionally, aging processes, particularly UV/H₂O₂ accelerated aging, significantly enhanced biodegradation by altering surface properties such as fragmentation and surface oxidation. For example, 10 days of UV/H₂O₂ aging increased the biodegradation rate constant (k, day⁻¹) from 0.0022 to 0.0096 for 20 μ m MPs. These findings suggest that PLA MPs subjected to natural weathering conditions may

degrade more rapidly than under controlled laboratory conditions, emphasizing the importance of understanding environmental factors in predicting the fate of biodegradable MPs.

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3.06.P-We242 Investigating the Properties and Quantities of Submicrometer Particles and Nanoplastics Released from PET and PLA Teabags

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Food contact materials (FCMs) are predominately made from polyethylene terephthalate (PET), due to its useful properties as a flexible, light weight, and heat-resistant material. In recent years, a shift to polylactic acid (PLA) has occurred as it is biobased and industrially biodegradable. A major concern regarding plastic FCMs is the observed release of micro- and nanoplastics as well as oligomers, for which human health risks are largely unknown. This study aims to fill an analytical gap by quantifying and characterizing the properties of submicrometer particles released from teabags when steeped in boiling water. Using the same experimental setup, leachates from PET and PLA teabags are produced, enabling a direct comparison of the released particles. The study has a specific focus on the detailed analysis of released oligomers, through chemical analysis and through their solubility in different solvents. These experiments will distinguish solid nanoplastic particles from (agglomerated) oligomers, measuring size ranges and concentration. To address this challenge, complementary analytical methods are used, including Fourier-Transform Infrared (FTIR) spectroscopy to determine the composition, Scanning Electron Microscopy (SEM) to observe the surface structural changes, Nanoparticle Tracking Analysis (NTA) determining particle size and concentration, and Liquid Chromatography Mass Spectrometry (LC-MS), identifying the oligomers, such as linear or cyclic, and their relative abundance. We expect a release of particles and hypothesize that in strong solvents most of the oligomers are dissolved and only few to no actual nanoplastics will be detected. The study will contribute to the mechanistic understanding of particle release from plastics, with methodological procedures that can also be extrapolated to other product categories such as household consumer products and synthetic textiles. This study will also contribute to a broader understanding of the release from nanoplastics and oligomers, paving the way for investigations of their environmental fate and potential human health and environmental risks.

3.06.P-We243 Identification of Biodegradable Plastic Fragmentation through Physical Abrasion in Freshwater

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Biodegradable plastics are increasingly being produced and consumed globally as alternatives to conventional plastics, with their market share steadily rising. However, while these plastics are designed to decompose effectively under specific temperature conditions in treatment plants, such conditions are rarely met in most natural environments, making complete degradation challenging. Similar to conventional plastics, biodegradable plastics undergo physical and chemical weathering, eventually breaking down into microplastics that persist in the environment. Although several researches have been conducted on the toxicity of biodegradable microplastics, there is a notable lack of studies addressing their environmental fate. Therefore, this study aims to investigate the physical weathering mechanism of biodegradable plastics within the laboratory scale. Polymer chips ($1 \times 1 \times 0.2$ cm) were exposed in the bottles that contains gravel and deionized water for a period of 90 days using roller and mixer device to simulate the flow. Five types of biodegradable plastics were tested: polylactic acid (PLA), polybutylene adipate terephthalate (PBAT), polybutylene succinate (PBS), polyhydroxybutyrate (PHB), and poly(3-hydroxybutyrate-co-3-hydroxy valerate) (PHBV). For comparison, two types of conventional plastics, polyethylene terephthalate (PET) and polystyrene (PS), were also tested. The physicochemical properties of the resulting microplastics were characterized using several analytical methods: field emission scanning electron microscopy (FE-SEM), Fourier transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS), while the remaining polymer chip were characterized with atomic force microscopy (AFM), X-ray diffraction (XRD), and Brunauer Emmett Teller (BET) surface area analysis. This study provides critical insights into the degradation mechanisms of biodegradable plastics in freshwater environments.

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3.06.P-We244 Degradation of Biodegradable Plastics

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Decades of extensive production and use of conventional plastics have led to their accumulation in the environment. Bioplastics (bio-based and/or biodegradable) have been promoted as a more sustainable alternative. However, the literature reveals conflicting conclusions regarding their suitability and environmental impact. A primary area of dispute concerns their biodegradability and the conditions necessary for proper degradation. In real-world settings, biodegradable plastics may not degrade as rapidly or efficiently as laboratory tests indicate. Here, we describe three activities that were conducted to address these issues: (1) a systematic literature review to explore the current level of knowledge, (2) a degradation test on consumer products made from biodegradable plastics was conducted based on an identified knowledge gap in the current literature, and (3) development of a novel oil-based method for extracting aged bio-microplastics from compost matrices. The review covered the degradation of biodegradable plastic in waste management environments (e.g., compost, sludge, or landfill) and the open environment (e.g., seawater, freshwater, or soil). The results highlight challenges in comparing and quantitatively analyzing data on plastic degradation due to methodological variations, including differences in testing methods, materials, and quantification strategies. Moreover, several research gaps and limitations exist. Notably, there is a need to intensify research on polyhydroxyalkanoates (PHAs), polybutylene adipate terephthalate (PBAT), and polybutylene succinate (PBS) to match the level of polylactic acid (PLA) and starch-based plastics. A degradation test was carried out on commercially available biodegradable plastics under simulated industrial composting conditions according to ISO 20200. The results indicated disintegration degrees between 75-100 % for all tested biodegradable plastic products, with five of ten reaching complete disintegration within 90 days. Finally, a method was developed to extract microplastics from these compost samples using oleoextraction. The outcomes of these studies offer valuable insights, particularly in guiding discussions about the prospective role of biodegradable plastics within society. Based on our findings, essential knowledge and data gaps will be highlighted, and suggestions will be provided regarding the direction of future research to assess their role as alternatives to conventional plastics.

3.06.P-We245 Toward Standardization in Microplastics Research: Development of an Extraction Protocol from Compost for ISO

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The current economic model is producing plastics in large quantities, and despite our best efforts to recycle and reuse them, some still end up in the environment. With the potential to have negative impacts on flora and fauna, with the potential to be harmful to humans as well. These plastics age and fragment over time, generating microplastics, which vary in color, shape, and physicochemical properties. These differences in properties between different particles challenge their treatment as only one type of contaminant. Besides that, despite knowing that microplastics are present everywhere, there is still no standard method for their extraction and characterization from environmentally complex matrices. This is why we are in the process to standardize a method for the extraction and characterization of microplastics from compost, with a plan to have it finalized by 2027 or earlier. This standard will allow for reproducible and homogenized results, allowing the comparison of findings between different studies and laboratories. The developed extraction protocol consists of deagglomeration, organic matter removal, and density separation. The organic matter removal is done by Fenton oxidation, and the density separation employs calcium chloride. Not only would it target the extraction of fossil fuel-based non-degradable microplastics, but also fragments from biodegradable polymers. Fragments from biodegradable plastics could be present during the initial stages of the composting process but are not expected to be found in final composting products. To achieve the extraction of microplastics, the extraction protocol, under standardization, does not apply harsh conditions. Current results show that the herein developed method has high recovery efficiencies for PBAT microparticles without affecting non-degradable plastics and has negligible effect on the size of biodegradable ones. For the characterization of the particles, we are using a micro-Raman system that employs a 785 nm laser and a particle finder/analyzer software to automate the process. Currently, an interlaboratory study is ongoing to test and validate the presented extraction protocol. Furthermore, we aim to sample final compost products from industrial composting plants in Austria and Germany, comparing plants serving small and large communities.

3.06.P-We246 On the Fragmentation of Soil-Biodegradable Mulch Film: Assessment of Interim Particle Size Distributions and Modelling for Half-Life Predictions

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Soil-biodegradable mulch films are an innovative solution that not only promotes plant growth, but also helps to reduce plastic accumulation in agriculture, leaving no persistent microplastics behind due to complete polymer mineralization during biodegradation.

Biodegradation of polymers is not a homogeneous process, and several factors influence the fragmentation of biodegradable polymers, such as microbial colonization, the polymer (blend) composition and shape. The formation of defects, peninsula structures and increasing embrittlement during biodegradation leads to interim fragment formation, which is an inevitable part of the biodegradation process and cannot be avoided. To gain a mechanistic understanding of copolymer blend fragmentation and biodegradation process, we studied biodegradation of a soil-biodegradable mulch film in soil, according to ISO 17556. A validated extraction technique, which is used for isolation of fragments of biodegradable plastics from soil was coupled with IR microscopy analysis to monitor fragment number, shape, size, and identity during biodegradation. The generated data was used for parameterization of the open-source FRAGMENT-MNP model. In addition, we determined the molar mass distribution at different biodegradation time points and assessed the ratio of hydrolyzed polymer to remaining polymer.

The combination of these methods allows for the comparison of fragmentation mechanisms of different types of copolymer blends and enable the prediction of the timepoint where the total particle counts of different size classes peak during the biodegradation process. Modeling approaches, further enable the prediction of half-lives of interim fragments, which will depend on the polymer type and environmental conditions. The revealing conclusion is that although interim fragments are formed during the biodegradation process, they will fully biodegrade until mineralized into CO₂ and biomass.

3.06.P-We247 Degradable or Not – Field Study, Laboratory Experiment and Farmer Survey on Biodegradable, Conventional and Oxo-Degradable Plastics in Northern Climatic Conditions

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Plastic mulches reduce the need of pesticides and irrigation, but cause soil contamination with plastics. To reduce the harm, biodegradable plastics have been developed. Biodegradable mulch films are not collected from the fields after use, but mixed with the soil to decompose. However, the decomposition rate depends on environmental conditions.

We studied plastic residues in agricultural fields after use of conventional and biodegradable plastics in Finland, in northern climatic conditions. Also, degradation in warm and cold temperature were followed in laboratory and a survey for farmers was conducted.

In the field study, the highest concentration of microplastics (20 µm-5mm) was observed at sites with oxo-degradable films. Biodegradable plastics in this size fraction were not analysed. In the fraction larger than 2 mm, the highest concentrations were found at sites with biodegradable mulch films for multi-season use, being about 100 times higher than at the sites with conventional polyethylene (PE) mulch films. In the survey for the farmers, 94% of the respondents announced to collect PE plastic fragments actively from soil (n = 66), whilst the corresponding share was only 12% for biodegradable plastics (n = 17). This may explain the low number of PE fragments found in the fields. The long degradation time of biodegradable plastics was also detected in the laboratory experiment. After 21 months of incubation, only slight signs of biodegradation in 21-22°C and no degradation in 1-3°C was detected.

These results imply that degradation of biodegradable mulching films can be slow in northern environmental conditions and that also biodegradable plastics can contribute to plastic pollution in agricultural soils. The environmental conditions should be taken into account in test standards and degradation criteria. In addition, product development is needed to find suitable materials for different environmental conditions.

The degradation of biodegradable mulching films in different climate zones is further studied in the project PAPILLONS in field conditions. This will give us valuable information on the factors affecting the degradation process in real environment.

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3.06.P-We248 Environmental Behavior and Fate of ¹⁴C-Poly Lactic Acid in Soils

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With the increasing global production of plastics, transitioning to a circular model has become a priority. Bioplastics, derived from renewable sources, present a promising alternative to traditional plastics by potentially reducing CO₂ emissions and dependence on fossil fuels. However, challenges persist regarding their recyclability and environmental fate. Understanding plastic degradation, particularly in complex environments like soil, is hindered by multiple variables, including polymer properties and environmental conditions, necessitating advanced techniques for precise evaluation.

The application of Carbon-14 (¹⁴C) tracing in plastic degradation research offers a significant advantage by enabling the tracking of polymer decomposition pathways and the quantification of degradation products. Traditional biodegradation studies often overlook the fate of residual polymers, focusing solely on evolved CO₂. In contrast, ¹⁴C labeling provides comprehensive insights into the remaining polymer fractions and their interactions within the environment.

Poly(lactic acid) (PLA) is a biodegradable polymer that widely used for different applications due to its composability and potential to reduce dependence on fossil fuels. Previous polymers degradation studies demonstrate that if a polymer is not inherently prone to degradation, or if the environment lacks degrading agents the process is extremely slow. Conventional plastics, such as polyethylene (PE), degrade at a rate of less than 1% per year, whereas bioplastics can degrade completely within a few months. Based on this, we expect the degradation of PLA to occur as rapidly as other bioplastics, given favourable environmental conditions.

To advance this understanding, we have developed a laboratory-scale synthesis procedure for producing ¹⁴C-labeled PLA. This modified synthesis pathway optimizes PLA production by addressing challenges such as the presence of unstable stereoisomers and impurities. The resulting ¹⁴C-labeled PLA will be utilized in soil degradation and leaching studies, focusing on two soil types: sandy and silty. Conducted over 180 days to 12 months, these experiments will assess the impact of temperature, soil humidity, and organic manure on PLA degradation. The evolved CO₂ will be quantified using Liquid Scintillation Counting (LSC).

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3.06.P-We249 Chemical Contaminants in Biodegradable Products: A Comparative Study of Per- and Polyfluoroalkyl Substances and Heavy Metals in the Netherlands and New Zealand

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Biodegradable products offer a viable solution to plastic waste, particularly for single use disposable plastics that are often entering the environment via littering, such as plastic bags, food packaging and coffee cups. However, these products contain chemical additives whose identity and corresponding environmental impact remain largely unexplored. Chemical additives are of particular concern in biodegradable products as their release into the environment may be accelerated via biodegradation of the product.

This project aims to quantify the amount of per- and polyfluoroalkyl substances (PFAS) and heavy metals in biodegradable products from both the Netherlands and New Zealand. Currently, regulation of biodegradability and compostability is much less rigorous in New Zealand than it is in the European Union. Furthermore, the difference in types and concentration of additives used in these biodegradable products between the two countries is unknown.

To address this knowledge gap, a comprehensive chemical analysis was conducted on ten selected biodegradable products from each region. Heavy metals were quantified via inductively coupled plasma-mass spectrometry (ICP-MS) following acid digestion. Solvent extraction was performed prior to ultra-performance liquid chromatography-high resolution mass spectrometry (UPLC-HRMS) analysis for the quantification of PFAS. Additionally, pyrolysis-gas chromatography-mass spectrometry (Pyr-GC-MS) was used to determine polymer composition. This revealed how the polymer composition influenced the types of additives present.

Our study revealed that many of the biodegradable products contained PFAS, including one composed of bagasse which was certified compostable. This study challenges the common perceptions of biodegradable products as being inherently environmentally benign by examining if their chemical profiles support these claims. This research also sheds light on the influence of regional policies and regulations on the use of chemical additives in biodegradable products, while identifying discrepancies between regions. Ultimately, this study can guide regulatory bodies in shaping future policies to ensure that biodegradable and compostable products meet high standards for both public safety and environmental protection.

3.06.P-We250 Laboratory to Field Scale: Ecological Relevance of Laboratory Tests for Environmental Reliable Ecotoxicity Assessment of Polymers

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Polymeric compounds are included into various agrotechnical products. Given that synthetic polymers and modified natural polymers are subject to REACH, biopolymers can be regarded as important substitute components. Due to their natural origin, they remain unregulated, even though their complex molecular structure led to increased accumulation potential in the environment. As regulatory requirements and hazard evaluation concepts are missing, potential ecotoxicological impact is unknown. Our main aim was therefore to develop a systematic ecotoxicological hazard assessment encompassing terrestrial and aquatic toxicity screening for biopolymers. In order to ensure the reliability of the data collected and prove the ecological relevance, a combination of laboratory tests and field studies has been utilized.

We focused on alginate, cellulose fibers and active char formulated in a seed coating with sugar beet. The treated soil was incubated under laboratory conditions in glass vessels (without seed) and under field exposure in small-scale lysimeters (with seed) with drainage system for leachate collection. The components were applied as single substances (1000 mg/kg) and as cryomilled formulations (1 and 3 coatings/2.5 kg). Additionally, seed coatings (1 and 3 coatings/2.5 kg) were exposed (field exposure). Leachate was analyzed for its NPOC content to determine leaching of the substances and formulations. In case of leaching, aquatic ecotoxicity tests with algae (OECD TG 201) and daphnids (OECD TG 202) would be conducted. Soil samples were taken after 28 and 100 days of exposure so far to evaluate substance and formulation impact on microbial functionality. Therefore, we conducted ISO 15685 (Potential nitrification) and ISO 20130 (Extracellular enzymatic activities) test.

The results indicated stimulatory effects of biopolymers and formulations on enzymatic activities after 28 days both under laboratory and field exposure with comparable stimulatory tendencies. After 100 days, these tendencies were not visible anymore. Inhibitory impacts were determined for active char treatment after 28 days on enzymatic activities as well as potential nitrification and after 100 days on extracellular enzymes. Laboratory results overestimated the effects observed under field exposure, indicating that the laboratory approach is a conservative and protective tool for risk evaluation.

3.06.P-We251 The Impact of Fossil and Biobased Microplastics on Human Cells

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Plastic pollution is one of the most significant environmental challenges of the twenty-first century, with microparticles now found in virtually all ecosystems. Humans are exposed to these particles through three primary routes: ingestion, absorption, and inhalation, and the potential effects largely depend on the composition, shape, and size of the particles. While most research on micro- and nanoplastics focuses on spherical or rod-shaped particles, with polystyrene (PS) being the most commonly studied polymer, the most widely produced plastics are polypropylene (PP) and low-density polyethylene (LDPE).

Additionally, there has been a rise in the production of biobased polymers, which degrade faster than traditional fossil-based plastics in an effort to mitigate the harmful effects of conventional plastics. However, the impacts of these newer materials remain largely unknown.

To better understand the effects of micro- and nanoparticles on human health, five types of plastic three from fossil sources (LDPE, PS, and PP) and two from biological sources (PLA and PHB) were mechanically degraded and separated by size using stainless steel mesh filtration and the fraction below 1.67 µm was chosen. Human cell lines from the liver (HepG2) and intestine (HCT116) were used as biological models and exposed to eight different concentrations of each plastic type and size range, starting from 1.28 µg/L to 100 mg/L. Cellular viability was assessed at three time points (24, 48, and 72

hours) using the MTT assay. In general, HCT116 cells showed greater sensitivity to the plastics than HepG2 cells, with toxicity being similar between fossil-based micro- and nanoplastics, but biobased present a higher impact than biobased microplastics. Our findings highlight the importance of further investigating the impact of novel biobased and biodegradable polymers on human health.

3.06.P-We252 Screening of the Environmental Safety of a Novel Biomaterial for Use as Alternative for Plastics

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Bioplastics are products with enhanced biodegradability and/or of biobased source whose growing production has the potential to replace conventional petroleum-based plastics. Natural biopolymers such as chitosan and cellulose are the most abundant on the planet and can be used for bioplastic production. Here, a newly-developed chitosan-methylcellulose bioplastic was investigated for its environmental safety in terms of its disposal after use in terrestrial environments as compost. For this, ecotoxicological tests using three higher plant species exposed to different concentrations of the tested bioplastic were ran. Bioplastics were mixed in standard soil at 0.1, 1, and 10% (w/w) concentrations, and parameters of seed emergence, root growth and shoot growth were measured after 3 and 6 days after seed incubation in dark at 25±1°C. The value for effective concentration to observe 50% of effect (EC50) was also estimated. There was a significant decrease in seed germination in seeds exposed to the 10% bioplastic treatment (avg. 8.3%) when compared to the control (avg. 98.89%). Previous studies have shown that plant seeds exposed to conventional plastic particles can have delayed seed emergence, but this could not be verified here as experiment was terminated at day 6. We hypothesize that delays in seed emergence might be related to reduced water availability caused by the bioplastic hygroscopic nature, and this is being currently tested. Root growth and shoot growth followed a similar pattern as seed emergence, with many zero values for root/shoot length at the 10% bioplastics treatment. EC50 values varied among species, and the dose-response curves highlight the datapoint gaps between treatments of 1%-10% bioplastics which calls for further experiments with a refined range of concentrations. In summary, the tested chitosan-methylcellulose bioplastic material showed no significant effects on seed emergence, root length, or shoot length in tested plant species at concentrations up to 1% bioplastic in soil (w/w). There appears to be a transient effect at a 10% concentration that can be related to changes in water availability caused by the bioplastic addition, and further tests are underway to confirm this. This study adds to the potential for using biobased materials to replace persistent, toxic plastics and for safely disposing the tested bioplastic in terrestrial environments as compost.

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3.06.P-We253 The Effects of Microplastics Derived from General Plastics and Biodegradable Plastics on *Daphnia Magna*

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The pollution of the oceans by plastics are one of the world's major environmental problems. In particular, there are concerns about the ecological effects of microplastics, which are the small pieces of polymers. In addition, although biodegradable plastics are said to be one solution to the problem of plastic waste in environment, there is no doubt that biodegradable plastics can also become the source of microplastics. There are many studies on the environmental behavior of these materials, but almost no examples of them having a negative impact in the actual environment, and the only reports we have are of laboratory tests, i.e., ecotoxicity tests. Furthermore, the test methods and endpoints used in these tests are diverse, and there is no standardized test method.

We have partially improved standard ecotoxicity testing methods and conducted *Daphnia* toxicity tests using microplastics of various sizes. We conducted a *Daphnia* test on microplastics derived from general plastics (PE, PP, PS) and microplastics derived from five types of biodegradable plastics obtained from manufacturers, and found that they showed different endpoints depending on their size. We also report on the differences in adsorption capacity and ecological impact of microplastics derived from biodegradable plastics and general plastics when exposed to polycyclic aromatic hydrocarbons.

3.06.P-We254 Characterization and Potential Toxicity of Environmentally-Aged Bioplastics

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The global bioplastic production is expected to increase from 2.18 million metric tons in 2023 to 7.43 million metric tons in 2028. With bioplastics increasingly positioned as alternatives to traditional plastics, questions remain whether they truly biodegrade and if they can leach harmful additives into the environment. To assess environmental fate and risks of bioplastics, characterization of environmentally aged bioplastics is needed. In this study, three bioplastic drinking straw materials, poly lactic acid (PLA), polyhydroxyalkanoate (PHA), polyhydroxybutyrate (PHB), and a starch-composite bag, and two controls (polyethylene and paper) were considered. Items were exposed in California ocean water and soil environments for 64 weeks. The paper straw fragmented quickly, losing 75% of its initial weight in the first 8 weeks. In comparison, PHA, PLA, and PHB lost 25-50% of their initial weight in that time. Importantly, fragmentation alone does not equate to biodegradation; materials may break apart but persist as microplastics. To differentiate surface and structural changes in the materials, attenuated reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) and scanning electron microscopy (SEM) tracked functional group shifts and morphological changes on the material surfaces. Spectral shifts can indicate whether there was biodegradation of the materials, and morphological changes and potential formation of micro- and nano-plastic particles. Additionally, targeted liquid chromatography and mass spectroscopy (LC/MS) was used to determine the occurrence of common plastic additives over time. A targeted analysis of phthalates, bisphenol-A, and 4-nonylphenol was conducted to determine the release of these common plastic additives from the bioplastic materials. Evaluation of bioplastic persistence in the environment is necessary to mitigate the global plastic pollution. This study provides insight into bioplastic end-of-life scenarios to inform public and policy decision-making.

3.06.P-We255 A Comparison Between Biodegradable and Conventional Mulch Films: Uptake and Impact on Marine Ecosystem Processes

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Biodegradable alternatives to conventional plastics are being developed largely in response to the impact of plastic pollution. This is most prevalent for products that are likely to end up in the natural environment, for example agricultural mulch films. Biodegradable plastics are designed to degrade under certain conditions (e.g., elevated temperatures, low pH, etc). In the natural environment, biodegradables are more persistent, thereby posing a risk to biota and ecosystems. Here we use a flow-through mesocosm system to examine the fate (i.e., uptake and distribution within ecological compartments) and impacts on marine ecosystem processes (i.e., microbial community structure, nutrient flux, benthic-pelagic coupling, bioturbation) of microplastics over a three month period. Microplastics comprised cryo-milled (<500 µm) biodegradable (PBAT/PLA) and conventional (polyethylene) mulch film. Mesocosms were seeded with benthic macrofauna with differing functional traits (vertical conveyors, biodifusers, surface modifiers) and dominant pelagic copepods. Distribution: Abiotic (i.e., sediment, water, fluff layer) and biotic (i.e., invertebrates) samples were analysed by pyrolysis coupled to gas chromatography and mass spectrometry (Py-GC-MS), using chemical markers to detect the specific polymers in biodegradable and polyethylene films. Microbial community impacts: After three months exposure, prokaryotic communities differed between control, biodegradable and polyethylene treatments in the sediment and water column, indicating the added particulate load altered microbial composition in both compartments. Invertebrate communities: Exposure to both treatments resulted in shifts in plankton community composition. There was no effect of treatment on macrofaunal biodiversity, however biomass was significantly reduced when exposed to polyethylene. Benthic-pelagic coupling: Exposure to polyethylene microplastics resulted in copepods producing significantly smaller and slower sinking faecal pellets than controls. Bioturbation: In the polyethylene treatment, we observed shifts in the conveyance of nutrients and particles at sediment depths of 2-6 cm. Further, nutrient levels (e.g., nitrites, phosphates) in treatment sediments differed from controls. Our novel mesocosm exposure system highlights how biodegradable and conventional microplastics can become distributed via abiotic and biotic processes, with substantial impacts on ecosystems.

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3.06.P-We256 It Depends Where it Ends - Understanding the Biodegradation Performance of Biodegradable Plastic Polymers Under Variable Environmental Conditions

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Short-chain-length polyhydroxyalkanoates (scl PHA) are readily biodegradable under suitable laboratory conditions. This makes them good reference materials also for field studies on biodegradable plastic polymers. However, several variables influence the performance of PHA in marine, freshwater and soil systems. In addition to temperature as the most important factor, the small-scale heterogeneity of environmental matrices and geographical locations must also be taken into account. Both area loss of films and surface erosion of thicker test specimens are suitable for assessing biodegradation in the environment if precautions are taken against physical forces and premature losses. Pre-weathering had little or no effect on the biodegradation of PHAs. The biodegradation rates of different co-polymers under different environmental scenarios from Central European agricultural soil to the deep sea are presented. In addition, we show how the results of laboratory, mesocosm and field studies can be made comparable through statistical modeling, how this enables predictions about the lifetime of products made from PHAs in the open environment, and how this can be incorporated into a life cycle analysis (LCA) and thus into the product environmental footprint (PEF).

3.06.P-We257 The Double-Edged Sword of Biodegradability: Lifecycle Carbon Impacts of Bio-Based Polymers vs. Fossil-Based Polymers

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Bio-based materials are often perceived as climate-neutral, but this assumption requires careful examination. This study conducts a lifecycle scenario analysis of biopolymers made from wood specifically lignin and cellulose nanofibrils, along with common fossil-based polymers (e.g., PET) and PLA to assess their carbon footprints. Results reveal a broad spectrum of climate impacts ranging from carbon-positive to carbon-negative for the woody biopolymers. In contrast, fossil polymers show lower variability in carbon footprints but lack carbon-negative lifecycle scenarios. The variability in the woody biopolymers' footprints was influenced by their end-of-life (EoL) scenarios. A no emissions EoL scenario assumes wood derivatives are not biodegradable and release no CO₂ or CH₄ at EoL, potentially resulting in negative carbon footprints. In contrast, the carbon footprints for landfilling scenarios are the highest owing to the assumed biodegradation and atmospheric emissions of biogenic CO₂ and CH₄. Thus, the incineration scenario for biopolymers, resulting in only biogenic CO₂ emission at EoL, leads to a lower lifecycle carbon footprint vs. landfilling. The EoL possibilities for fossil non-biodegradable polymers are limited (landfilling without degradation, incineration, or recycling), only allowing for CO₂ emissions and not the more potent CH₄. Hence, while wood derivatives may offer negative carbon footprints, their worst lifecycle scenario has almost thrice the carbon footprint of the worst PET use case.

These results as computed through LCA methods therefore counterintuitively underscore that the non-biodegradability of fossil plastics, which has led to the crises of macro- and micro-plastic pollution, is seemingly favorable from a climate perspective. Strategies to improve the durability and recyclability of biobased products would reduce emissions but may compromise biodegradability. This highlights a potential trade-off in environmental objectives, where priorities like reducing plastic pollution and addressing climate change may demand conflicting EoL strategies. The current focus on carbon emissions has therefore overshadowed the broader motivations behind establishing a bioeconomy. Existing LCA tools may not be ideal to capture all sustainability advantages of biopolymers impacts from, e.g., reliance on intensive agriculture for biopolymer feedstocks are effectively incorporated, but not environmental benefits like reducing plastic pollution.

3.06.P-We258 Bio-Based Polymers in Thermosetting Coatings: Insights and Future Directions From the EU SAFERCOAT Project Towards More Sustainable Solutions

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Polymers are fundamental in modern society due to their versatility and widespread applications, from everyday products to specialized industrial uses, such as protective coatings. However, the environmental impacts of traditional synthetic coatings, including dependence on fossil resources, limited biodegradability, and disposal challenges, have driven the development of bio-based thermosetting coatings (bioTC). BioTC, formulated with a minimum of 30% bio-based building blocks, such as lignin and vegetable oils, are increasingly explored as sustainable alternatives due to their biodegradability potential. Although bioTCs show promise, their thermal stability, chemical resistance, and mechanical durability are limited compared to traditional coatings, which restricts their use in high-demand applications such as maritime protective coatings. To address these issues, nanotechnology might be used to immobilize active ingredients (e.g., polymer-hydrolyzing enzymes) in smart nanomaterials (e.g.,

layered double hydroxides). The EU SAFERCOAT project proposes this innovative approach to extend the lifespan of TC coatings by controlling the undesired early leaching of active ingredients, offering ecotoxicological benefits compared to commercial soluble forms. The project aims to develop smart or stimuli-responsive nanoadditives to be incorporated in novel multifunctional and eco-friendly bioTC to enhance the polymer's (bio)degradability at the end of service life. Despite these advancements, challenges remain, including achieving high-performance standards, minimizing toxicity, and promoting end-of-life degradation. While bio-based coatings offer eco-friendly potential, their (bio)degradability can be limited to specific conditions. Moreover, although research on the environmental impacts of bio-based polymers is growing, most studies focus on bioplastics, leaving a critical gap in the assessment of the toxicity and hazard of bio-based coatings and their leachates in marine environment. Therefore, this study aims to provide a comprehensive review of the state-of-the-art in terms of bio-based coatings, identifying existing gaps and limitations and addressing the possible future directions towards the development of truly eco-friendly and high-performance thermosetting coatings with enhanced biodegradability at the end of service life.

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3.07.A Wide-Scope Target and Non-Target Screening Strategies for Enhanced Chemical Coverage in Environmental Monitoring and Chemical Exposome Assessment

3.07.A.T-01 Assessing Extraction Methods for Chemical and Effect-Based Analysis of Environmental Contaminants in Breast Milk

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Population growth has increased chemical production and human exposure to xenobiotics that may impact health. Under the "One Health" approach, which links human, animal, and environmental health, exposomics investigates cause-effect relationships of exogenous compounds. This study aimed to develop an extraction method for detecting a broad range of xenobiotics in breast milk using an effect-based approach to assess their biological impact. An existing method for human plasma combining passive equilibrium sampling (PES) with solid-phase extraction (SPE) was adapted for high-throughput analysis of breast milk samples from volunteers, both spiked and unspiked. However, neurotoxicity bioassay results revealed precipitation-associated interferences in the final extracts, indicating that the initial protocol inadequately removed matrix components such as lipids. A simplified solvent precipitation method with 0.22 µm PTFE filtering was introduced to enhance fat removal. Polar and semi-polar contaminants were analyzed with liquid chromatography-high resolution mass spectrometry (LC-HRMS), and hydrophobic compounds with gas chromatography-high resolution mass spectrometry (GC-HRMS). A neurotoxicity bioassay based on the inhibition of neurite outgrowth in differentiated SH-SY5Y cells evaluated the effects of the contaminants.

Results highlighted challenges in integrating chemical analysis and bioassays within the same workflow. The adapted protocol failed to sufficiently remove fat content, likely causing the cytotoxicity observed during neurite outgrowth measurement. Meanwhile, solvent precipitation led to compound loss and insufficient matrix removal: recovery rates in the chemical analysis were below 60% for over 80% of compounds, while effect recoveries reflected matrix effects rather than neurotoxic activity. Further optimization is required to reduce lipid content and enhance compound extraction before scaling up to larger sample sets. Incorporating a saponification step into the plasma protocol may improve lipid removal. Additionally, a non-target screening prioritization would provide a more comprehensive overview, reducing the risk of overlooking neurotoxic compounds excluded from the target analysis.

3.07.A.T-02 CPxplorer and Silicone Wrist Bands as Rapid Tools for Evaluating Human Exposure to Polychlorinated Alkanes

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Chlorinated paraffins (CPs) are complex industrial mixtures primarily made of polychlorinated alkanes

(PCAs, $C_nH_{2n+2-x}Cl_x$) but also byproducts. CPs are used in metalworking fluids, lubricants, flame retardants, and PVC additives. Despite their extensive utility, PCAs are considered persistent, bioaccumulative, and toxic, leading to the international ban of short-chain CPs (C10-13, SCCPs) in 2017. Medium- and long-chain CPs (C14-17, MCCPs; C17-30, LCCPs) remain unregulated due to lack of exposure and risk assessment data. This results from the analytical challenge of PCA quantification, which lacks suitable standards and must address the strong overlap of millions of isomers. Accordingly, there is a knowledge gap on human exposure to PCAs. Dermal contact, and ingestion of indoor dust and organic films (IOFs) are some of the main pathways for human exposure to PCAs. Silicone wristbands (SWBs), made of polydimethylsiloxane, are non-invasive, flexible and cost-effective tools for monitoring personal exposure to chemicals like pesticides and flame retardants. However, due to analytical challenges with PCAs, only one study has explored their use for PCA exposure assessment. Our study evaluates SWBs as a personal sampling tool for measuring indoor PCA exposure and proposes the use of the open-source Skyline software together with an in-house developed app (CPxplorer) for rapid data processing and quantification of PCAs. In this study, 32 volunteers wore pre-cleaned SWBs for seven days during June 2024 in Sweden. The data from the SWB study was evaluated and quantified using Skyline and CPxplorer. Preliminary results showed the highest levels for ?PCAs-C14-17 (37.0 $\mu\text{g/g}$) in the SWBs, followed by ?PCAs-C10-13 (7.8 $\mu\text{g/g}$) and ?PCAs-C18-30 (3.9 $\mu\text{g/g}$). In combination with our previous data from indoor dust and IOF, these PCA patterns suggested indoor dust as a key exposure contributor. Moreover, higher PCA levels were found in households built after 2017, aligning with the SCCP ban. Children showed higher exposure than adults, likely due to greater dust contact. Dermal exposure estimates were measured at 12.4, 43.7, and 0.2 $\mu\text{g/kg bw/day}$ for ?PCAs-C10-13, ?PCAs-C14-17, and ?PCAs-C18-30, respectively. These results indicated that SWBs, combined with data from indoor matrices, offer a promising method for assessing PCA exposure. The study also validates the use of Skyline and as efficient tools for PCA analysis and quantification.

3.07.A.T-03 Exploring Metal-Organic Framework-Coated Blades for Direct and High-Throughput Screening of Xenobiotics in Urine

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The analysis urine is one of the most desirable matrices to assess the exposure of humans to a vast variety of xenobiotics that could lead to hazardous and undesirable effects on our health. From an analytical point of view, the application of human biomonitoring programs in hospitals poses a huge challenge since the analysis of urine usually requires a high working load which results in the delay of the results difficulting the decision making. In this way, high-throughput screening approaches such as ambient ionization mass spectrometry (AIMS) that allows both the preconcentration, clean-up and fast analysis of the samples could provide a step forward to the implementation of biomonitoring programs of complex matrices in the medical field. To expand such biomonitoring tools, in this work, metal-organic framework (MOF) coated blades were tested for the target and suspect screening analyses of xenobiotics in urine by coated blade spray-high-resolution mass spectrometry (CBS-HRMS).

First of all, three different MOF-coated blades were synthesized and properly characterized. After that, both the extraction and desorption of selected analytes was carefully optimized by choosing a fast extraction procedure as well as the most suitable MOF-coated blade type, desorption solvent and voltage. Under optimal conditions, the CBS-HRMS methodology was validated achieving limits of detection down to 0.03 ng/mL as well as a good precision (RSD < 30%) and linearity ($R^2 > 0.99$). To enhance the throughput and the reliability of the analysis, a semi-automated acquisition consisting of 3 desorptions in 5 min and an adequate suspect screening workflow was proposed to sort out the challenges of carrying out these approaches with AIMS. The screening method was then applied to the analysis of urine samples collected from volunteers at the University of the Basque Country (Spain) showing the capabilities to both fastly quantify and identify xenobiotics in this complex biofluid and its potential for the medical field.

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3.07.A.T-04 Prioritizing Candidate Structures in Non-Targeted LC/ESI/HRMS Analysis by Combining Machine Learning Predictions

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Non target screening with liquid chromatography high resolution mass spectrometry (LC/HRMS) has rapidly gained popularity in detecting and identifying environmental hazards. The primary tool for structural elucidation of the detected LC/HRMS features is MS2 spectra. However, the ambiguity in these spectra results in long lists of candidate structures, necessitating further investigation to identify the correct one. Empirical analytical information predicted by machine learning (ML) models holds promise in reducing the candidate list and increasing the confidence in certain candidate structures by deprioritizing the candidates that mismatch experimental results.

To evaluate the efficiency of different types of empirical analytical information, we assess five ML prediction models for their ability to reduce the candidate lists. Specifically, these included: (1) two retention time (RT) prediction models, MultiConditionRT and Retention Time Index (RTI); (2) an ionizability prediction model; (3) a sodium adduct prediction model, and (4) the similarity between experimental and in-silico MS2 spectra from CFM-ID. The selected models were used to prioritize the structural candidate lists generated from SIRIUS+CSI:FingerID. Various thresholds were applied to determine whether candidates should be deprioritized, allowing for the evaluation of each model's performance. Additionally, the potential of combining information from these models was explored. The results indicated that RT predictions could provide the best performance in terms of eliminating the greatest number of candidates (efficiency) while avoiding filtering out the correct structures (accuracy). Nonetheless, the results from the preliminary tests for combining the predictions revealed an undesirable rate of correct candidate removal and highlighted the shortcomings of the harsh filtering implementation. A probabilistic approach to prioritize structure candidates is being considered to address the current challenges and facilitate structure elucidation in NTS analysis. In particular, the proposed method will leverage Bayesian learning, treating the predictions from ML models as expert knowledge to prioritize the candidate and increase confidence in structure elucidation.

3.07.P-Mo226 What's in Our Consumer Products? Target, Suspect, and Non-Target Screening

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Various studies have examined the presence of potentially harmful additives in consumer products like food contact materials and children's toys. These additives, including flame retardants (FRs), plasticizers, and other substances, are intentionally added to improve the performance of plastics. However, because most additives are not tightly bound to the polymer, they can leach out during use, potentially exposing users to toxic chemicals. While the European Union has regulations to control certain harmful substances in plastic toys, such as PBDEs, phthalates, and BPA, these chemicals still enter the market due to poor recycling practices or lack of regulation in manufacturing countries.

To assess whether consumer products purchased in the Netherlands and the U.S. were contaminated with regulated or unregulated compounds, a selection of 65 food contact items, hair accessories, kitchen utensils, and toys was analyzed for the presence of various flame retardants, phthalates, and plasticizers. In the second phase of the project, non-targeted and suspect screening was conducted to explore additional substances present in these consumer products.

The results showed widespread contamination with hazardous chemicals. TBBPA was the most commonly detected flame retardant, followed by BDE-209. In 70% of the plastic products, BDE-209 exceeded the EU's unintentional trace contaminant limit of 10 mg/kg, and more than six different FRs were detected in 60% of the products. DecaBDE replacements like DBDPE and TTBP-TAZ were also found. High levels of phthalates and plasticizers were found in toys, including five substances classified as substances of very high concern (SVHCs) by ECHA, which are linked to hormone disruption, reproductive toxicity, and cancer. Legal limits were exceeded for four of these substances, with DBP surpassing the limit by 200 times. The plasticizer DEHTP was found in twelve toys, with concentrations up to 50% of the total weight.

The data processing for the non-target and suspect screening is ongoing. The detected features were annotated using publicly available Spectral Libraries (MassBank EU and MassBank North America) and the plastCHEM Analyte List. Additionally, the polymer type of each consumer product was determined to examine any patterns related to the type of polymer used.

3.07.P-Mo227 Can the Use of Menstrual Products Contribute to Chemical Exposure?

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Menstrual products, fundamental to maintaining hygiene, preventing infections and ensuring participation in our society during menstruation, have been shown to contain chemicals with potential toxic effects, like phthalates (PAEs) and per- and polyfluoroalkyl substances (PFAS). Since the vulvar and vaginal tissues in contact with these products have high chemical absorption capacity, the use of these products might contribute significantly to human exposure. However, the studies investigating this exposure pathway are still scarce and limited to few products and chemicals. In this study, we wanted to expand our understanding of human exposure through the use of menstrual products by quantifying 4 classes of chemicals of concern for human health, PAEs, organophosphate esters (OPEs), non-phthalate plasticizers (NPPs) and PFAS, in both single-use and reusable menstrual products. A total of 41 menstrual products (10 sanitary pads, 8 panty liners and 9 tampons, 4 reusable sanitary pads, 4 menstrual panties and 6 menstrual cups) were analyzed for 9 PAEs, 17 OPEs, 10 NPPs 21 PFAS. To estimate the contribution of dermal contact with menstrual products to human exposure, the estimated daily intakes (EDI) were calculated assuming the worst-case scenario of 100% release of the chemicals from the products and 100% absorption through the skin. The combination of PFAS and plasticizers analysis showed that a wide variety of chemicals can be present in menstrual products. All types of menstrual products analyzed had detectable concentrations of PAEs, OPEs and NPPs, while PFAS were only detected in reusable menstrual products. Differences in detection frequencies and concentrations were observed across different products potentially due to differences in product design. Reusable sanitary pads showed the highest concentrations of PAEs and OPEs and menstrual panties showed the highest PFAS concentrations. The highest NPPs concentrations were observed in single-use sanitary pads and panty liners. For PAEs, OPEs and NPPs, worst-case scenario estimates showed that using these products might contribute significantly to human exposure. As an example, for reusable sanitary pads the EDI for OPEs (median: 213 ng/kg bw/day) was above estimates for dietary exposure (62-103 ng/kg bw/day), which is considered the main OPEs exposure route. These results highlight the need investigate the release of these chemicals from menstrual products to the skin to provide more realistic estimates.

3.07.P-Mo229 Chemical Background in Blood Microsampling Devices by Non-Target Chemical Exposomics

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Blood microsamplers are of interest for exposomics applications as they enable quantitative and high-frequency self-sampling of individuals outside of the clinic. These devices, being simple to use and minimally invasive, may be deployable by laypeople and shipped at room temperature, facilitating high-throughput sampling at cohort-scale to comprehensively characterize the chemical exposome in human blood. One challenge in their implementation is the chemical background they can introduce, which may lead to false-positives when characterized by liquid-chromatography coupled to high-resolution mass-spectrometry (LC-HRMS). Here, the chemical background of several popular dried- and liquid-blood devices was assessed by sampling high-purity water, contrasting the resulting total ion current (TIC) intensities, number of non-target molecular features detected, the peak area distributions of all features, and their chemical characterization.

Using LC-MS grade water as a blood proxy, we assessed the chemical background of 8 commercially available devices: Capitainer® B and B50 (Capitainer AB, Solna, Sweden); Neoteryx Mitra® VAMS® (Neoteryx, Torrance, CA, USA); Tasso-M20 and 3 versions of Tasso+ (Tasso, Inc., Seattle, WA, USA); and TAP® II Lithium Heparin (LiH) (YourBio Health, Inc., Medford, MA, USA). After sampling, the water was left to dry overnight. Then, each device (n = 4 replicates) was extracted with either 60:40 ACN:H₂O or 60:40 MeOH:H₂O with a 15 min sonication, followed by centrifuge filtration (0.2 µm, Nylon). Analysis was performed by reversed-phase chromatography (Acquity BEH C18, Waters), and mass spectral data were acquired in full scan (m/z range 80 920) and by data-independent MS/MS in ESI±, using an Orbitrap Exploris 480 (ThermoFisher Scientific). Raw data were pre-processed using the open source software MS-DIAL (v4.9).

Our study showed that chemical background varies greatly between devices, with some specific contaminations (e.g., nicotine in Capitainer B50) and some being shared across multiple devices (e.g., diphenyl phosphate in all Tasso devices and Mitra VAMS). Overall, 3 samplers have a comparably low chemical background: Neoteryx Mitra VAMS, Tasso+ Plasma, and TAP II LiH. Other devices may still be more appropriate considering sampling volumes and ease of use. Investigating the chemical background of

microsamplers facilitated by the use of collection and field blanks will be crucial for chemical exposome studies in human blood.

3.07.B Wide-Scope Target and Non-Target Screening Strategies for Enhanced Chemical Coverage in Environmental Monitoring and Chemical Exposome Assessment

3.07.B.T-01 Echoes of Exposure: Unveiling Chemical Exposome in Human Reproductive System with A Non-Target Analysis Approach Using High-Resolution Mass Spectrometry

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Parental exposure to synthetic chemicals prior to conception may have critical impacts on fertility and reproduction. Therefore, it is essential to establish highly sensitive analytical methods to investigate the occurrence of xenobiotics and their metabolites at trace level in the reproductive fluids from females and males. In this study, a sophisticated solid-phase (micro)extraction (μ SPE) method covering a broad spectrum of chemicals was applied to prepare analytical samples from various biological matrices, including serum paired with follicular fluid (FF) from 12 female participants and blood paired with seminal fluid (SF) from 12 male participants, all receiving fertility treatment. The chemical exposome was characterized by a non-target analysis (NTA) approach using Orbitrap-based high-resolution tandem mass spectrometry (HRMS) platform hyphenated to capillary- (cLC)/nano-flow liquid chromatography (nLC). The NTA workflow was optimized and applied using commercially available software Compound Discoverer 3.3 to decipher the acquired HRMS data, including spectra analyses, structural elucidation and annotation via in-house and public available databases matching for identifications. Isotope-enriched analogues of xenobiotics were added to every sample to enable recovery calculation, matrix effect evaluation, and feature identification.

By applying the NTA workflow, we could assign unambiguous molecular formulas to 234 and 83 prioritized features detected in nLC- and cLC-HRMS, respectively. Further spectral inspections achieved identifications of a total of 117 compounds with high confidence (? level 2). Notably, 21 xenobiotics were detected in FF or SF for the first time, indicating that they can cross blood-follicle/testis barriers and highlighting their potential risks for fertility. Moreover, multiple PFAS were detected in FF but not in SF samples, addressing gender-specific accumulation in the reproductive system and possible implications for infertility. In conclusion, significant preconception exposure to various xenobiotics was observed in both males and females undergoing fertility treatment. Multiple endocrine disruptors have been shown to cross blood-follicle/testis barriers, suggesting their potential relevance to male and female fertility impairments. The results provide knowledge needed for the construction of realistic human low-dose mixture exposure for the study of its putative impacts on human fertility.

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3.07.B.T-02 Chemical Characterization of the Prenatal Exposome Through the Analysis of Cord Blood Samples Using Liquid Chromatography Coupled to Trapped Ion Mobility Spectrometry-High Resolution Mass Spectrometry

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Humans are exposed daily to a myriad of xenobiotics through diet, inhalation, and direct contact. Collectively, these lifelong exposures constitute the chemical exposome. However, exposure during pregnancy and early childhood is particularly concerning, as it can lead to significant adverse health outcomes. To comprehensively characterize this exposome, chemical profiling via high-resolution mass spectrometry serves as a powerful tool for uncovering the intricate chemical landscape affecting human health.

This study focuses on identifying xenobiotics in cord blood serum samples from pregnant women, aiming to assess the prenatal chemical exposome. Over 500 samples were collected from the Barcelona Life Study Cohort (BiSC) and processed using a standardized protocol involving deproteinization, evaporation to dryness, and reconstitution in MeOH:H₂O (50:50), targeting polar to moderately polar non-volatile xenobiotics. The analysis was conducted using liquid chromatography coupled with trapped ion mobility spectrometry and high-resolution mass spectrometry (LC-TIMS-HRMS), employing optimized data acquisition modes (bbCID and PASEF). More than 2,500 xenobiotics were screened in a targeted manner, identifying >100 chemicals across various classes along with their metabolites, in concentration ranges from high ng/mL to low mg/mL. These included many pharmaceuticals, with those associated with labor at detection frequencies >80% (e.g., bupivacaine), food-derived compounds with high detection frequencies (around 90-100%) (e.g., caffeine and its metabolites), plastic additives in varying detection frequencies, from 10 to 90% (such as phthalate metabolites and bisphenols), pesticide metabolites at low detection frequencies (e.g., the captan metabolite 1,2,3,6-tetrahydrophthalimide), as well as highly frequent industrial chemicals (e.g., certain PFAS) among some others.

The findings revealed that specific exogenous chemicals can cross the placental barrier, potentially posing risks to the developing fetus. The study demonstrated the ability of advanced instrumentation, such as LC-TIMS-HRMS, in combination with generic sample preparation protocols, to be leveraged for broad targeted chemical coverage in the investigation of fetal exposure to xenobiotics. Future efforts will incorporate expanded suspect screening strategies to identify additional chemicals, further refining the holistic definition of the chemical exposome during prenatal stages.

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3.07.B.T-03 Single Drop, Multiple Findings: Feasibility of Dried Blood Spots for Broad Chemical Coverage in Exposomics/Metabolomics

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Dried blood spots (DBS) are a established sample at newborn screening program for the screening of metabolic diseases. As such, it holds great potential as a simple, non-invasive and alternative sample for the assessment of chemical exposure. Nevertheless, the systematic evaluation of extraction efficiency and matrix effects for a large set of exposure compounds, along with the evaluation of additional compounds detected by non-targeted data analysis has not yet been conducted. Here we present the results of a 20-min LC-HRMS methodology for non-targeted exposomics research on DBS samples, including detailed analytical performance for 223 highly diverse exposure chemicals. Four distinct extraction protocols were compared by using pre- and post-spiked DBS prepared and extracted in a quantitative manner. The selected optimal extraction protocol presented promising results for a large number of compounds, with around one third of all compounds fulfilling a narrow analyte acceptable range (80-120%) and more than 50% falling into a broader acceptable recovery range (50-150%). Reproducibility was also acceptable, with median RSD for recovery at 18%. Similarly, matrix effects presented a median value of 72%, with

RSD at 14%. Untargeted data analysis was used for the annotation of endogenous molecules such as amino acids at high confidence levels, showcasing the potential for combined exposomics/metabolomics research. Ongoing experiments and data analysis include the application of the method to a small pilot study with DBS collected from six smoking and non-smoking donors as well as the estimation of limits of detection by using DBS samples spiked a low concentration level. In the future, the method should be applied to a large-scale screening programme for the evaluation of exposure levels and trends in such a vulnerable population.

3.07.B.T-04 Chlorinated Paraffins in Novel Plant-Based Foods

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In recent years, plant-based (PB) diets (such as veganism or vegetarianism) have become increasingly popular in Western countries. Along with this popularity, the demand and market for novel plant-based foods (NPBFs) grew rapidly in the last years, offering a large variety of often ultra-processed alternatives. The degree of food processing has been associated with potential contamination of different chemicals, among these also chlorinated paraffins (CPs). CPs are complex mixtures of polychlorinated n-alkanes (PCAs). They vary in chain-length and chlorine content (30-70%) and can be divided into short-chain (SCCP, C10-13) restricted as persistent organic pollutants (POPs), medium chain (MCCP, C14-17) and long-chain (LCCP, C>17) CPs. These compounds are of high concern for the environment due to their persistent, toxic and bioaccumulative properties and their high production volume.

In this study, we investigated the occurrence and patterns of CPs (C8-36, C13-30) in NPBFs (n = 50) purchased in Europe using liquid chromatography coupled to high-resolution mass spectrometry. Homologue identification and integration were performed with the R-based open-source software CP-Seeker v1.1. The quantification of each CP class was conducted by chlorine-content calibration using mixtures of SCCPs, MCCPs and LCCPs (C18-20). Exposure and risk assessment was performed for three assumptive dietary scenarios; flexitarian, vegetarian and vegan diet.

?SCCPs could be detected in 56% of the samples with values ranging from <LOQ to 27 ng/g wet weight (ww), ?MCCP had a detection frequency (DF) of 88% (<LOQ-133 ng/g ww) and ?LCCPs had a DF of 24% (<LOQ-19 ng/g ww). PB-cheese was the most contaminated food category for ?SCCPs (<LOQ-27 ng/g ww) and LCCPs (<LOQ-13 ng/g ww), ?MCCPs were higher in PB-cold meat (<LOQ-133 ng/g ww). PB-cheese consists mostly of coconut oil and CP contamination has been associated with lipid content, which could explain the elevated levels found in the samples. The consistently high ?MCCP levels in PB-cold meat samples suggest a specific similar processing technique, which could introduce this contamination. Compared with conventional foods, NPBFs had higher CP concentrations than their animal-based homologues. Dietary exposure and risk assessment showed a limited risk for all scenarios. However, based on the elevated levels in NPBFs and their increasing popularity more studies are recommended to provide a comprehensive food safety assessment.

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3.07.B.T-05 Pyrolysis-GC-MS for Quantitative Analysis of Micro- and Nanoplastics in Liver Samples for Exposome Assessment

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Micro- and nanoplastics (MNPs), defined as solid particles ranging between 5000 µm and less than 1 µm, are contaminating food, water, air, and eventually accumulating within the human body. Accurate quantification of MNPs in human biological samples is essential for assessing human exposure and understanding the potential health implications of these pollutants. Previous research has confirmed the

presence of MNPs in human blood and other biological tissues. The liver is a particularly relevant organ for evaluating MNP levels, as it plays a central role in filtering, storing, and eliminating toxins, offering valuable insights into bodily exposure. However, detecting MNPs in human samples is an analytical challenge due to the complex nature of biological matrices and the limited availability of sensitive detection methods.

Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS) is a powerful technique for MNPs analysis, providing mass-based quantitative data with high specificity and sensitivity. This makes it especially suitable for analysing complex matrices like liver tissue. In this study, we developed and validated a Py-GC-MS-based analytical method to measure the mass of six common plastic polymers poly(methyl methacrylate), polyethylene, polypropylene, polystyrene, polyvinyl chloride, and polyethylene terephthalate in liver samples. Method development employed chicken liver as a model matrix, creating a strong foundation for applications to human liver samples. The digestion procedure was carefully optimized to completely remove the organic matrix while preserving the target particles. Strict quality control protocols were established to ensure analytical accuracy and to minimize contamination from lab equipment, airborne particles, and reagents. The detection of MNPs in human liver tissue can enhance our understanding of how these particles are absorbed, accumulate, and potentially impact health over time. The method was successfully validated for liver samples, confirming its reliability. This work emphasizes the importance of robust digestion protocols tailored to complex biological matrices, facilitating accurate MNPs quantification in health-related research. The developed method represents a significant step forward in human biomonitoring of plastic pollutants and evaluating MNPs exposure through human tissue analysis.

3.07.C Wide-Scope Target and Non-Target Screening Strategies for Enhanced Chemical Coverage in Environmental Monitoring and Chemical Exposome Assessment

3.07.C.T-01 Non-Target Analysis of Micropollutants in Drinking Water From China: Profiles and Prioritization

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Micropollutants are increasingly detected in drinking water. Due to their persistence, bioaccumulation and toxicity, they pose potential risks to human health and aquatic ecosystems even at low concentrations. Low concentrations and the complexity of the water matrices present great challenges to the analysis of micropollutants in drinking water. With narrow scope of target pollutants, incomplete toxicity data, and the inadequacy of conventional monitoring methods, many contaminants may be overlooked. Advanced non-target analysis offers a promising solution to these limitations, providing a more comprehensive understanding of water contamination and aiding in the development of more effective treatment and risk assessment strategies. A nontarget screening strategy was performed on drinking water samples from 12 different provinces in China, using capillary-flow liquid chromatography coupled to high-resolution Orbitrap mass spectrometry. Across all samples, more than 40,000 chemical features was annotated, and after strict filtering and prioritization, 189 organic micropollutants with high confidence levels (level 1-3) were annotated, including pharmaceuticals, pesticides, flame retardants, and emerging and legacy per- and polyfluoroalkyl substances. Differences in the profiles of contaminants in different regions and different types of samples were observed. With combination of an deep-learning endocrine-disrupting chemicals predictor, the study provide a comprehensive understanding of micropollutants cocktails in drinking water.

3.07.C.T-02 Anthropogenic Profiling of European Surface Water Using Feature-Based Molecular Networking and Non-Target Analysis

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In 2023, a large study was undertaken to understand the spatial and temporal exposure of 54 active pharmaceutical ingredients across European surface water systems. A total of 280 sites across 31 European countries were sampled in each season (summer, autumn, winter, and spring) and analysed using a targeted analysis approach. To expand on this, a comprehensive analysis of the total anthropogenic contamination profile of European surface waters was performed using non-target chemical analysis methodologies. Complimentary nanoflow liquid chromatography high-resolution mass spectrometry platforms were utilised to obtain chemical information on thousands of detected features across a wide chemical space. Network-based data analysis was utilised for elucidation of compounds, revealing the presence of hundreds of xenobiotics and transformation products. In silico tools were used to increase the annotation rate of unknowns by structure, class, and formula predictions. Semi-quantification was used to determine chemical concentrations across the European fresh water systems.

This is the first study of its kind to apply non-targeted chemical analysis to surface waters at such a large spatial scale. Thousands of annotated features were present in European surface waters many of these from anthropogenic sources. The findings demonstrate the power of combining large-scale monitoring campaigns with non-targeted analysis approaches to understand chemical contamination of the natural environment.

3.07.C.T-03 Advanced Micropollutant and Contaminant (CEC) Wastewater Surveillance for Strategic Targeted Sampling

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The increasing prevalence of contaminants of emerging concern (CECs) in water systems, coupled with diminishing water resources, underscores the need for innovative monitoring and treatment strategies at wastewater treatment plant (WWTP) outlets, for chemical identification. Traditional evaluation approaches often rely on interval-based sampling for a narrow range of chemicals, overlooking many micropollutants and their transformation products. Given the temporal variability of CEC discharges and their dynamic presence in water networks, real-time, targeted monitoring strategies are essential.

This study demonstrates the utility of ToxMate online biomonitoring, which leverages organism avoidance behavior as a biomarker for non-targeted, effect-based effluent characterization. Laboratory experiments have established a behavioral database of avoidance responses for over 40 micropollutants, including pesticides, pharmaceuticals, and hydrocarbons. Using functional data analysis (FDA), this data forms "behavioral fingerprints," enabling classification of avoidance response based on chemical nature of the pollution. Early results reveal structural similarities among overlapping micropollutants, suggesting a pathway for real-time identification of persistent or unknown CECs. Furthermore, the ToxMate device is equipped with an automatic grab sampler, only triggering samples for analysis upon micropollutant surge events. Fieldwork at a WWTP site in northwestern France, operational since January 2022, highlights the practical application of this approach. Over two months, 14 micropollutant surge events triggered automated grab sampling for wide-scope chemical analysis. Notably, control samples collected outside alert events exhibited minimal concentrations of all dosed chemicals, reinforcing the sensitivity of the biomonitoring system. The results also show that events with similar behavioural profiles show striking resemblance in their chemical profile, dosed for over 803 chemicals in a targeted chemical approach. These findings suggest that coupling behavioral fingerprinting with high-resolution mass spectrometry (HRMS) can narrow the scope of chemical analysis, facilitating targeted monitoring and real-time alerting. By identifying critical moments of CEC surge, this strategy offers WWTPs the ability to optimize contaminant management, refine advanced treatment processes, and potentially minimize environmental risk.

3.07.C.T-04 Quantification Without Reference Standard: How Semi-Quantitative Tools Performed in Water Sample?

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The quality of water (ground, surface and tap) is a major societal issue. Both public and private authorities are dedicated to upholding quality standards in accordance with regulatory mandates. The list of parameters subjected to sanitary control may evolve with the identification of new substances (active substances, metabolites) or new toxicity information. Therefore, it is essential to be able to provide answers regarding the identity and concentration of these substances to assess information on environmental pollution. The unavailability of standards (not commercially synthesized, expensive costs) raises questions about confidence levels attributed to compounds identification and semi-quantification. Semi-quantitative approaches aim to estimate compound concentration based on its properties and signal

intensity without using the associated reference standard.

This work introduces the development of a quantitative and semi-quantitative screening method for organic contaminants in water. This method is based on Liquid Chromatography coupled with High Resolution Mass Spectrometry (HRMS). Analyses were carried out with Data Independent Analysis (DIA) acquisition mode to perform targeted, suspect and retrospective analysis by querying internal and external databases. The objective was to be able to identify a maximum of organic contaminants through two acquisitions (positive and negative ionization)

To characterize method performance, limits of quantification were determined for more than 450 molecules. In addition, several multi-residue proficiency tests (>100 compounds) were conducted to demonstrate the applicability of the method. The results were promising with a majority of the expected molecules detected; the remaining ones can mainly be analysed using other separation techniques (HILIC, IC, GC). The database query made it possible to identify molecules without a reference standard. For these, semi-quantification tests based on existing methodologies (2D structure similarities, ionization efficiency prediction) were carried out to evaluate the performances of these approaches. Furthermore, semi-quantification will be done on real water sample, from the intake and output of drinking water treatment plants, to evaluate the level of contamination of these resources. Future work will involve testing semi-quantification approaches on other samples and food matrices to better assess the capacities of this approach.

3.07.C.T-05 Non-Target Screening in Suspended Particulate Matter and Biota Using GC-HRMS

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Chemicals are indispensable to modern life and industry, yet they also present a significant risk to human health and the environment. Despite the registration of over 350,000 chemicals worldwide, more than 120,000 of them remain unidentified, which complicates monitoring efforts.

The aim of this project was to develop a method for the detection of both known and unknown apolar substances in suspended particulate matter (SPM) and fish fillet. Within this process, the establishment of a discrimination free non-target screening for the detection of spatial and temporal trends in samples from the German Environmental Specimen Bank was prioritized.

Methods for sample extraction and clean-up, GC-MS analysis and data analysis were developed and validated using a mixture of 76 compounds. For SPM ultrasonic extraction and silica clean-up was used. For fish file an additional gel permeation chromatography step was established.

LODs in SPM for a variety of different substances, such as PAHs, PCBs, UV-filter, pesticides etc., were determined to be between 0.2 and 5 ng/g for the most substances. The workflow enabled the detection of all spiked substances, resulting in a qualitative recovery of 100%.

The initial findings demonstrate that the newly developed methods are able to detect a broad range of known contaminants, such as PAKs and phthalates, as well as previously unrecognized environmental pollutants such as Plastic Additive 11 (Octadecyl-3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionat). This highlights the potential of the method to detect future contamination trends and to respond to new types of pollutants at an early stage.

This project makes an important contribution to environmental monitoring due to its capacity to perform a comprehensive analysis of the occurrence of contaminants and demonstrates how new and potentially harmful substances can be identified and assessed at an early stage. The workflow can be used complementary to non-target LC-MS methods to extend the polarity range, thus representing a valuable contribution to a more comprehensive monitoring.

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3.07.P Wide-Scope Target and Non-Target Screening Strategies for Enhanced Chemical Coverage in Environmental Monitoring and Chemical Exposome Assessment

3.07.P-Mo226 What's in Our Consumer Products? Target, Suspect, and Non-Target Screening

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Various studies have examined the presence of potentially harmful additives in consumer products like food contact materials and children's toys. These additives, including flame retardants (FRs), plasticizers,

and other substances, are intentionally added to improve the performance of plastics. However, because most additives are not tightly bound to the polymer, they can leach out during use, potentially exposing users to toxic chemicals. While the European Union has regulations to control certain harmful substances in plastic toys, such as PBDEs, phthalates, and BPA, these chemicals still enter the market due to poor recycling practices or lack of regulation in manufacturing countries.

To assess whether consumer products purchased in the Netherlands and the U.S. were contaminated with regulated or unregulated compounds, a selection of 65 food contact items, hair accessories, kitchen utensils, and toys was analyzed for the presence of various flame retardants, phthalates, and plasticizers. In the second phase of the project, non-targeted and suspect screening was conducted to explore additional substances present in these consumer products.

The results showed widespread contamination with hazardous chemicals. TBBPA was the most commonly detected flame retardant, followed by BDE-209. In 70% of the plastic products, BDE-209 exceeded the EU's unintentional trace contaminant limit of 10 mg/kg, and more than six different FRs were detected in 60% of the products. DecaBDE replacements like DBDPE and TTBP-TAZ were also found. High levels of phthalates and plasticizers were found in toys, including five substances classified as substances of very high concern (SVHCs) by ECHA, which are linked to hormone disruption, reproductive toxicity, and cancer. Legal limits were exceeded for four of these substances, with DBP surpassing the limit by 200 times. The plasticizer DEHTP was found in twelve toys, with concentrations up to 50% of the total weight.

The data processing for the non-target and suspect screening is ongoing. The detected features were annotated using publicly available Spectral Libraries (MassBank EU and MassBank North America) and the plastCHEM Analyte List. Additionally, the polymer type of each consumer product was determined to examine any patterns related to the type of polymer used.

3.07.P-Mo227 Can the Use of Menstrual Products Contribute to Chemical Exposure?

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Menstrual products, fundamental to maintaining hygiene, preventing infections and ensuring participation in our society during menstruation, have been shown to contain chemicals with potential toxic effects, like phthalates (PAEs) and per- and polyfluoroalkyl substances (PFAS). Since the vulvar and vaginal tissues in contact with these products have high chemical absorption capacity, the use of these products might contribute significantly to human exposure. However, the studies investigating this exposure pathway are still scarce and limited to few products and chemicals. In this study, we wanted to expand our understanding of human exposure through the use of menstrual products by quantifying 4 classes of chemicals of concern for human health, PAEs, organophosphate esters (OPEs), non-phthalate plasticizers (NPPs) and PFAS, in both single-use and reusable menstrual products. A total of 41 menstrual products (10 sanitary pads, 8 panty liners and 9 tampons, 4 reusable sanitary pads, 4 menstrual panties and 6 menstrual cups) were analyzed for 9 PAEs, 17 OPEs, 10 NPPs 21 PFAS. To estimate the contribution of dermal contact with menstrual products to human exposure, the estimated daily intakes (EDI) were calculated assuming the worst-case scenario of 100% release of the chemicals from the products and 100% absorption through the skin. The combination of PFAS and plasticizers analysis showed that a wide variety of chemicals can be present in menstrual products. All types of menstrual products analyzed had detectable concentrations of PAEs, OPEs and NPPs, while PFAS were only detected in reusable menstrual products. Differences in detection frequencies and concentrations were observed across different products potentially due to differences in product design. Reusable sanitary pads showed the highest concentrations of PAEs and OPEs and menstrual panties showed the highest PFAS concentrations. The highest NPPs concentrations were observed in single-use sanitary pads and panty liners. For PAEs, OPEs and NPPs, worst-case scenario estimates showed that using these products might contribute significantly to human exposure. As an example, for reusable sanitary pads the EDI for OPEs (median: 213 ng/kg bw/day) was above estimates for dietary exposure (62-103 ng/kg bw/day), which is considered the main OPEs exposure route. These results highlight the need investigate the release of these chemicals from menstrual products to the skin to provide more realistic estimates.

3.07.P-Mo228 Untargeted Screening for PFAS Chemicals Using Comprehensive Feature Detection

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PFAS (poly/per-fluorinated aliphatic substances) are of increasing concern environmentally and the determination of PFAS levels within test samples is strategically important. According to the published literature, known PFAS chemicals, quantitated on the basis of reference standards, have been shown to

account for only 20-30% of the total organic fluorine in a sample. The remaining 70-80% is derived from other PFAS entities that are either commercial chemicals for which no reference standards are available, or downstream degradation/metabolism products of commercial chemicals with novel structures.

Methods

LC/HR-MS and LC/HR-IMS-MS data on a variety of representative environmental samples were acquired on Waters SYNAPT XS, SELECT SERIES Cyclic IMS and SELECT SERIES MRT spectrometers coupled with ACQUITY UPLC. Targeted screening was undertaken using a library of several hundred known PFAS chemicals, and during processing all detected components were evaluated against a list of common PFAS product ions and neutral losses. $^{12}\text{C}/^{13}\text{C}$ isotope ratios were also determined during peak detection and componentisation.

Preliminary data

We present outcomes obtained using a new software application, PFAS Targeter, which takes comprehensive advantage of the idiosyncratic properties of PFAS chemicals when compared with naturally occurring compounds. The approach uses class-specific product ions and neutral losses, mass defect, $^{12}\text{C}/^{13}\text{C}$ isotope ratio, trendlines on Kendrick plots and the distinctive relationships between both m/z and CCS, and between m/z and $^{12}\text{C}/^{13}\text{C}$ isotope ratio, in order to discriminate those components which are most likely to be PFAS-related from the matrix background. Given that many PFAS structural classes are inherently polymeric in nature, the software also iterates end group-monomer-end group signatures for common classes of PFAS, and uses these during processing to attempt to rationalise components which were not matched during targeted screening. The value of this approach to analysis of complex environmental samples is exemplified using both LC/MS and LC/IMS-MS data, and validated the use of ion mobility as an additional discriminator for PFAS in that greater enrichment of PFAS-probable components was obtained when ion mobility endpoints were included in component ranking and filtration.

3.07.P-Mo229 Chemical Background in Blood Microsampling Devices by Non-Target Chemical Exposomics

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Blood microsamplers are of interest for exposomics applications as they enable quantitative and high-frequency self-sampling of individuals outside of the clinic. These devices, being simple to use and minimally invasive, may be deployable by laypeople and shipped at room temperature, facilitating high-throughput sampling at cohort-scale to comprehensively characterize the chemical exposome in human blood. One challenge in their implementation is the chemical background they can introduce, which may lead to false-positives when characterized by liquid-chromatography coupled to high-resolution mass-spectrometry (LC-HRMS). Here, the chemical background of several popular dried- and liquid-blood devices was assessed by sampling high-purity water, contrasting the resulting total ion current (TIC) intensities, number of non-target molecular features detected, the peak area distributions of all features, and their chemical characterization.

Using LC-MS grade water as a blood proxy, we assessed the chemical background of 8 commercially available devices: Capitainer® B and B50 (Capitainer AB, Solna, Sweden); Neoteryx Mitra® VAMS® (Neoteryx, Torrance, CA, USA); Tasso-M20 and 3 versions of Tasso+ (Tasso, Inc., Seattle, WA, USA); and TAP® II Lithium Heparin (LiH) (YourBio Health, Inc., Medford, MA, USA). After sampling, the water was left to dry overnight. Then, each device ($n = 4$ replicates) was extracted with either 60:40 ACN:H₂O or 60:40 MeOH:H₂O with a 15 min sonication, followed by centrifuge filtration (0.2 μm , Nylon). Analysis was performed by reversed-phase chromatography (Acquity BEH C18, Waters), and mass spectral data were acquired in full scan (m/z range 80-920) and by data-independent MS/MS in ESI $\pm$, using an Orbitrap Exploris 480 (ThermoFisher Scientific). Raw data were pre-processed using the open source software MS-DIAL (v4.9).

Our study showed that chemical background varies greatly between devices, with some specific contaminations (e.g., nicotine in Capitainer B50) and some being shared across multiple devices (e.g., diphenyl phosphate in all Tasso devices and Mitra VAMS). Overall, 3 samplers have a comparably low chemical background: Neoteryx Mitra VAMS, Tasso+ Plasma, and TAP II LiH. Other devices may still be more appropriate considering sampling volumes and ease of use. Investigating the chemical background of microsamplers facilitated by the use of collection and field blanks will be crucial for chemical exposome studies in human blood.

3.07.P-Mo230 Effect-Directed Analysis of Human Cord Blood to Identify Potential Thyroid Hormone System Disrupting Chemicals

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The study of chemicals with endocrine-disruptive properties in human cord blood is crucial for understanding early-life exposure to environmental contaminants and their potential health effects. Traditional methods face challenges in identifying active chemicals within complex mixtures due to the diversity of compounds present. Effect-directed analysis (EDA) offers a solution by combining biological activity assessment with chemical profiling in a single workflow. This involves fractionating sample extracts, testing fractions in bioassays, and identifying chemicals in active fractions. Here, a high-throughput EDA was employed to examine the ability of chemical mixtures in human cord blood to compete with thyroid hormone (TH) for binding to transthyretin (TTR), a protein essential for TH regulation.

Cord blood samples from the Odense Child Cohort (500 boys, 250 girls) were extracted using lipophilic-hydrophilic balanced solid-phase extraction. TTR-binding activity was measured, and samples were pooled into four groups based on sex and activity (High/Low IC₁₀). Pooled extracts and their matching procedural blank were fractionated by high performance liquid chromatography (HPLC) coupled to a fraction collector (FractioMate). TTR-binding activity was measured in all fractions. Chemical profiling was conducted with non-targeted high-resolution mass spectrometry (Data Dependent Acquisition), using the same chromatographic conditions. Annotation of candidates was performed aligning different publicly available spectral libraries and a suspect list of chemicals of emerging concern to the extracted features. The analysis revealed sex-specific and within-sex variations in TTR-binding activity. Active fractions showed consistent patterns at the end of chromatographic runs, although some bioassay interferences were excluded. Ongoing alignment of bioassay data with chromatograms aims to identify key activity drivers. This study highlights the successful application of high-throughput EDA, enabling the prioritization of chemicals for identification and enhancing confidence in identifying drivers of endocrine disruption in early-life exposures.

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3.07.P-Mo231 Throughput Enhancement and Modelling Dimensionality Reduction by Sampling LC-HRMS Data

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Classification models are applied to various chemistry-related challenges, such as in wastewater treatment plant to determine which contaminants are present. Creating a robust classification model requires a large dataset with a limited number of descriptors. However, analysing many samples can be expensive and time consuming, and in the case of Liquid chromatography coupled with High Resolution Mass Spectrometry (LC-HRMS), the datasets generated per chromatogram are large. As a result, in reality, the data often include numerous features derived from only a few samples. While the feature reduction problem has been thoroughly investigated to identify the most prominent markers for classification, reducing the complexity of acquired data through compression can minimize the need for extensive feature reduction and potentially facilitate optimization of data acquisition and its processing. Instead of using the whole chromatogram, a part of the chromatogram (e.g., a peak list or chromatogram region of interest) could be representative enough to solve a classification problem. This approach can improve data processing and collection speed as well interpretability of the results, however, it is more prone to error since it represents a reduced data set resulting from preprocessing steps. Besides, different peak finding algorithms could be compared since they may result in different amounts of features. Therefore the investigation of the balance between data number of features and the amount of important information for classification should be investigated.

For this study, we explored various approaches to data (pre-)processing of LC-HRMS/MS data to identify the minimal information and resolution requirements necessary for accurate classification and marker detection. The various approaches include experiments with different widths of retention time bins, range of mass channels, and the use of compressed chromatograms.

By reducing the time and amount of data acquired while maintaining resolution, we can accelerate and improve the accessibility of classification and diagnostic tasks.

3.07.P-Mo232 Suspect Screening of Endocrine Disruptive Compounds (EDCs) in the European Environment

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Endocrine disruptive compounds (EDCs) represent a class of structurally diverse chemicals, emitted to the environment from multiple sources, that may interfere with human and animal hormonal systems and lead to adverse health effects. Identifying and quantifying EDCs in the environment is needed to better characterize the chemical mixture and exposure. However, due to the complexity of environmental matrices, the large number and variety of compounds, several analytical challenges and knowledge gaps still exist.

In this context, the Partnership for the Assessment of Risks from Chemicals (PARC) aims to develop the next-generation chemical risk assessment. A pilot study in the field of Environmental Monitoring aims to characterize and quantify EDCs in air, soil, surface water and biota by combining target chemical analyses, suspect screening and bioassays. Samples were collected throughout Europe considering different anthropogenic pressures. This presentation will focus on the suspect screening analysis.

From an initial aggregation of several lists of known or suspected EDCs, resulting in more than 7000 potential EDCs, a suspect screening list was created with relevant information for EDC identification. Standard operating procedures for sample collection and preparation were developed to ensure comparable results. Since the work involved several partners, quality standards were set to ensure that interpretation of the results would be feasible.

This collaborative effort of a dozen European institutes over the last year will be presented and lessons learned so far for a study of this scale will be provided. Analyses are currently being carried out on the 180 samples collected for all matrices and first results will be presented. Expected outcomes are the identification of EDC hotspots in Europe and identification of the most prominent compounds classes in different matrices and parts of Europe. Initial discussions of the potential origin and the environmental fate of relevant EDCs will be provided, based on their occurrence in the different environmental compartments.

3.07.P-Mo233 Usage of Liquid Chromatography and High-Resolution Mass Spectrometry to Detect and Monitor New Contaminants in Resources and Drinking Water

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In France, drinking water production is regulated through several parameters. The micropollutants are monitored with targeted methods, to detect and quantify their presence. While adequate for assessing treated water quality, this approach may overlook emerging pollutants, as only pesticides and their metabolites are regulated. High-Resolution Mass Spectrometry (HRMS) coupled with Liquid Chromatography (LC) enables non-targeted screening to detect compounds without preselection. Key steps in this process include optimized sample preparation and data treatment.

We applied a sample preparation method using Vacuum Evaporation Concentration (VEC), evaluated with a mixture of 195 compounds from various families, molecular masses, and physicochemical properties, to cover a wide part of the chemical space occurring in water samples. Evaporates were analyzed on an LC-qOrbitrap system, and data were processed with Thermo Compound Discoverer. Compounds were classified using an adapted Schymanski framework into identified, suspected, and features. Filters on analytical factors such as peak shapes were added to enhance data quality and confidence.

This method was applied to raw water resources (surface and groundwater) and treated drinking water from various locations in France and abroad. 99 compounds were identified based on retention time, exact mass, and fragmentation pattern, including pesticides (42%), drugs (31%), and their transformation

products. Additionally, 112 compounds were suspected and categorized into pesticides, drugs, and industrial or natural products. Based on occurrence, toxicity, and family type, 81 compounds were prioritized, and analytical standards were obtained for 67. Of these, 38 were confirmed. All of them were integrated into an internal database to reduce false positives in future analyses. To track ubiquitous versus sporadic pollution, monthly grab samples of the Seine River were collected from April 2023 to July 2024. This methodology identified 72 compounds and suspected 56, with 18 compounds detected consistently, including drugs like lidocaine, pesticides like methyl-desphenyl chloridazon, or industrial chemicals such as benzotriazole and its metabolite methyl-benzotriazole.

Non-targeted suspect screening complements targeted methods by detecting and identifying a broader range of compounds, guiding future targeted studies and monitoring.

3.07.P-Mo234 Setting the Baseline of Contamination by Emerging Pollutants in the Saronikos Gulf, Greece, Utilizing State-of-the-Art HRMS Techniques and Novel Chemometric Tools

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Comprehensive monitoring of environmental pollution is considered the basis for exploring the chemical imprint of human-related activities in the marine environment, which adversely impact its quality. The E.U. has enacted the Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD) to promote continuous scientific investigation and expedite regulatory bodies research towards preserving the marine realm.

Aiming to destress the Mediterranean Sea from chemical pollution, the E.U. has launched the RHE-MEDiation Lighthouse project. The characterization of the baseline of pollution close to areas considered as contamination hotspots is included in its framework. Wastewater treatment plants comprise major point sources of pollution in the vicinity of Saronikos Gulf. Assessing the quality of surrounding marine ecosystems sheds light towards WWTPs efficiency in wastewater stream cleanup processes. Saronikos Gulf is a heavily urbanized and industrialized area, while its two WWTPs of Thriasio and Psytallia cover more than half of the country's population in terms of wastewater management.

To determine as many emerging pollutants (EPs), along with their respective metabolites and transformation products (TPs) as possible, collected seawater and sediment samples were pre-treated via generic sample preparation protocols. Samples were analyzed utilizing novel LC-TIMS-HRMS workflows, aiming to determine EPs with a wide spectrum of physicochemical properties.

Preliminary results indicate that polar organic EPs, such as pharmaceuticals and plant protection products are mainly detected in seawater samples, while compounds with higher log P values, like industrial chemicals and long chain PFAS are generally concentrated in deep seawater samples and in sediments. Antiepileptic substance Carbamazepine and its metabolite 10-hydroxy-Carbamazepine and polar psychoactive substances Venlafaxine and Sulpride, along with their respective metabolites Amisulpride and o-desmethyl-Venlafaxine were determined in concentrations ranging between 0.235 and 10.4 ng/L in seawater samples. Sediments mainly contained semi- to non-polar compounds, like quinolone antibiotics Cinoxacin, Ofloxacin and Norfloxacin, as well as sulfonamide antibiotics Sulfadiazine and Sulfisoxazole, in concentrations levels ranging between 0.126 and 7.65 µg/kg d.w.

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3.07.P-Mo235 Investigating the Chemical Imprint of Human-Related Activities on the Red Sea Marine Ecosystem Utilizing Novel Complementary Mass Spectrometric Techniques

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Environmental monitoring of chemical pollution is deemed necessary to provide information about ecosystem quality. Anthropogenic chemicals such as pharmaceuticals, industrial chemicals, PFAS, PAHs, and PCBs end up in environmental substrates via different pathways. Upon reaching the marine environment, biotic and abiotic processes take place, producing metabolites and transformation products, suspected to cause even more potent effects than parent compounds, further degrading the marine environment.

Extensive urbanization and industrialization on the Red Sea coasts lead to unavoidable chemical encumbrance. The Kingdom of Saudi Arabia has launched the Marine and Coastal Environment

Protection Initiative to assess the current state of its coastal environment. Jeddah lagoons act as large banks of sediment formation, an environmental compartment which can be potentially burdened with pollutants with high log P values and volatile organic compounds, due to sorption and precipitation mechanisms. Besides sea bottom degradation, polluted sediments become potential sources of seawater recontamination, adding to the existing sources of pollution to which marine organisms are exposed to. Aiming to determine a wide variety of organic pollutants in collected sediments from Jeddah lagoons, generic sample preparation protocols and complementary analytical techniques were applied. Pollutants of various polarities and physicochemical properties were determined utilizing both GC-MS/MS and LC-HRMS techniques. HRMS-based wide-scope target screening was employed using an in-house developed dataset of more than 2,500 substances.

Preliminary results show that Jeddah lagoons are chemically encumbered by different groups of pollutants, based on different point sources in the vicinity. Antibiotics, psychoactive substances, as well as pollutants linked to industrial activity, such as PFAS were determined. GC-amenable compounds like hydrocarbons were detected, further indicating an evident chemical burden. Total aliphatic hydrocarbons concentrations ranged between 404 mg/kg d.w. and 8.90 g/kg d.w., while PAHs concentration levels ranged between 288 ?g/kg d.w. and 13.7 mg/kg d.w. Total PCBs and linear alkylbenzenes fluctuated from 0.100 to 15.6 ?g/kg d.w. and 173 ?g/kg d.w. to 41.8 mg/kg d.w., respectively. These results indicate the inevitable chemical encumbrance linked to anthropogenic activities and sewage disposal throughout the Red Sea area.

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3.07.P-Mo236 Investigating Fire Fighter and E-waste Handlers Exposure to PFAS Using Liquid Chromatography and Cyclic Ion Mobility Mass Spectrometry

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Polyfluorinated alkyl substances (PFAS) exposure is a potential contributor to increased cancer and disease in the human population. Firefighters and E-waste handlers have a high exposure to PFAS due to their occupational demands. Monitoring of PFAS levels in human biofluids helps gain understanding into exposure levels and pathways.

PFAS isomeric compounds can be challenging to efficiently separate using liquid chromatography (LC). Cyclic ion mobility (cIM) provides an added dimension of separation, enhancing peak capacity and collision cross section (CCS) values, which serve as a complementary identification descriptor. Using a 22 min reversed phase separation gradient, LC-cIM-MS (cIM resolution (R)~65-145) non-targeted analysis has been performed to analyse anonymised human serum samples of Ghanaian firefighters and E-waste handlers. Human serum sample extraction was performed using SPE 96-well μ Elution plates, containing a polymeric reversed-phase, weak anion exchange mixed-mode sorbent.

Typically, ? CCS < 1% has been observed compared to the PFAS library CCS values. For E-waste handler serum samples, PFAS, including, PFBS, PFOA, PFOS, 6:2 FTS and N-MeFOSSA (? CCS < 0.5%), have been identified. In Firefighter serum samples, PFAS, including, PFHxS, PFOS, PFHpS and PFNA (? CCS < 1%), have been identified. Additionally, we present identification of perfluoroalkyl carboxylic acids (PFCA) in human serum using ion mobility conformeric profile specificity, enabling highly specific identification at low intensities where product ions are absent.

LC-cIM-MS has been used to analyse a series of PFAS analytical standards comprised of structurally equivalent PFOA and PFOS branched isomers. Characteristic br-PFAS CCS values have been determined for branched PFOS and PFOA isomers, to extend PFAS library content. A comparison between br-PFAS travelling wave ion mobility (TWIM) CCS values, drift tube and trapped ion CCS measurements, ? CCS < 0.6% has been obtained. For PFOA characteristic isomer dimer CCS values have also been determined providing additional CCS fingerprint specificity. LC-cIM-MS can form a critical part of analytical strategy to enhance identification specificity of br-PFAS and provides an opportunity to correlate genotoxicity and environmental fate of br-PFAS. LC-cIM-MS is a highly specific non-targeted analysis strategy that can be used to identify known and unknown PFAS in complex samples.

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3.07.P-Mo237 The Occurrence of Contaminants of Emerging Concern in Swedish Landfill Leachate

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Landfill is one of the most common waste management methods, but it is not the final destination for many chemicals. Contaminants of emerging concern (CECs) are hazardous organic chemicals widely used in consumer and industrial products that can eventually leach into the environment from, among others, landfills. Before stricter waste management regulations were implemented in Sweden, a wide range of waste was deposited in landfills, highlighting the importance of investigating the occurrence and levels of CECs in order to fully understand their ecological and human health impacts. Non-target screening (NTS) and suspect screening (SS) using ultra high performance liquid chromatography-electrospray ionization-high resolution mass spectrometry (UHPLC-ESI-HRMS) are commonly used method for identifying unknown compounds in water samples. The goal for this study is to create a list of representative CECs in Swedish landfill leachate for future monitoring and quantification.

In our study, samples (untreated and treated leachate) were collected from five different Swedish landfills of different locations, age, and treatment methods. Both individual samples and pooled samples were prepared before analysis. Extraction was performed on homemade multi-layer Solid Phase Extraction (SPE) cartridges in order to capture a wide spectrum of CECs. Given the high sensitivity and selectivity of the UHPLC-ESI-Orbitrap instrument, analysis of pooled samples addresses some common challenges in NTS. For instance, the composition of pooled samples can help generating a suspect list of CECs representative of the Swedish scenario for later on application into individual samples, therefore significantly reducing total data processing time. Pooling samples can also address issues with different peak shape and shifted retention time due to varying matrix effects.

In this study, a total of 74 CECs were identified in the landfill leachate via NTS and included in a suspect list of CECs in Swedish landfills. Among the spotlighted CECs, 35 industrial chemicals, 21 pharmaceuticals, 10 PFASs, and 9 pesticides were detected. From them, 39 CECs were confirmed at level 1 and 35 were confirmed at level 2. Our findings underscore the diverse presence of CECs in landfill leachate, which aligns to previous studies.

3.07.P-Mo238 Improved Environmental Contaminant Identification using a Novel Dual-Ionization GC-EI&CI-TOFMS

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The accurate identification of environmental contaminants is essential for protecting public health and preserving ecosystems. Persistent organic pollutants (POP), such as dioxins and polychlorinated biphenyls (PCB), are of particular concern due to their long-term stability, bioaccumulation potential, and toxicity even at trace levels. Emerging pollutants like tire wear particles (TWP) and other contaminants such as pesticides and pharmaceuticals further exacerbate environmental challenges.

Traditional GC-MS approaches using EI offer reliable fragmentation data but often lack the molecular ion information needed for unambiguous identification. Chemical ionization (CI) enhances molecular ion detection while reducing the fragmentation necessary for structural elucidation.

To address these challenges, we introduce a novel dual-ionization GC-EI&CI-TOFMS system. This technique integrates the strengths of EI and CI within a single analysis, enabling robust fragmentation data alongside clear molecular ion signals. This dual-ionization approach enhances the identification of challenging contaminants providing a significant advancement in environmental monitoring and regulatory compliance.

Different environmental samples, e.g., soil extracts were investigated focusing on the identification of contaminants. For these studies a Bruker GC was coupled to an ecTOF (Bruker, Bremen, Germany). Various GC methods and sampling procedures were employed depending on the analytical need of the study. To generate the ideal molecular ion information different reactant ions were used for the chemical ionization process.

The results of the studies show that the EI&CI-TOFMS is feasible for standard procedures employed by routine laboratories. Whilst standard GC-MS methods mainly focus on target analysis, especially suspect screening and non-target analysis is enabled and improved by the EI&CI-TOFMS as detector. Especially when EI library hits are only accounted as fair with low corresponding probability, the additional CI information can be used to increase compound identification confidence. False positives from an EI-only approach can easily be identified and often correctly annotated. Furthermore, compounds not listed at all in libraries have a much higher confidence for identification using the combined EI&CI approach.

Additionally, using the accurate mass information and isotopic pattern fit on the molecular ion provided by CI often tentative identification can be derived.

3.07.P-Mo239 Non-Targeted Analysis of Contaminants of Emerging Concerns (CECs) in Consumer Food Packaging using Ultra-Performance Liquid Chromatography High-Resolution Mass

Spectrometry (UPLC-HRMS)

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Recent concerns have increased regarding the migration of substances from food packaging materials into food. Chemicals such as bisphenols, phthalic acid diesters, colorants, lubricants, and stabilizers are well-recognized as intentionally added substances. Additionally, non-intentionally added substances can also be present, these may include impurities and by-products generated during the packaging production process or at any stage of the material's lifecycle. These substances together with the intentionally added substances can pose migration risks into food, resulting in unforeseen contamination and potential health hazards. The comprehensive identification of these potential migrants is required to assess the safety of these packaging materials. The analysis of potential migrants is complex and challenging due to the numerous steps involved in the production of multilayer packaging, from raw materials to finished films. While targeted analysis using ultra-performance liquid chromatography mass spectrometry (UPLC-HRMS) is effective for detecting and quantifying certain intentionally added chemicals, it may not identify unknown substances. Therefore, non-targeted analyses are essential for enhancing and streamlining the comprehensive identification of these unidentified compounds.

In this study, 30 samples from various biodegradable food packaging materials, including straws, pizza boxes, and containers, were analyzed, among others, using non-targeted and suspect screening in both positive and negative ion mode. This analysis was conducted with (UPLC) coupled to quadrupole time-of-flight mass spectrometry. The identification and semi-quantification of unknown compounds were performed using patRoom, specifically focusing on poly- and perfluoroalkyl substances, phthalates, and bisphenols. The results obtained from the different food packaging materials were reported and compared between the Netherlands and New Zealand.

This research highlights the role of non-target analysis for identifying potentially overlooked substances present in biodegradable food packaging materials. The findings from this study significantly enhance the identification of chemicals present in food packaging materials, which is crucial for ensuring consumer safety. By improving the detection of both known and unknown compounds, the research contributes valuable data that can inform future studies and regulatory frameworks.

3.07.P-Mo240 Uncovering the Chemicals in House Dust

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Humans spend approximately 90% of their time indoors, increasing their likelihood of exposure to hazardous chemicals, many of which may accumulate in indoor environments. Young children are particularly vulnerable to higher exposures due to the frequent hand-to-mouth behaviors. Indoor dust, a complex matrix, contains a wide range of chemicals and is one of the exposure pathways for humans and a source of chemicals to the environment. The aim of this study was to annotated chemicals in house dust of different European countries.

In the EU-funded INQUIRE project, which aims to enhance indoor air quality, 200 house dust samples were collected in 8 European countries. Samples were analysed with liquid chromatography-high resolution mass spectrometry quadrupole time-of-flight (LC-HRMS-QTOF) and in addition with ion mobility HRMS using suspect and non-target screening (SNTS) approaches. A fast SNTS screening method was developed and optimized. A larger number of chemicals were detected, more than 20000. Regional differences of chemicals in house dust were found showing that for instance the UK had a distinct chemical profile from the other countries. Various groups of chemicals were identified such as lipids, steroids, insect repellents, fragrances, herbicides, and plasticizers, present at varying concentrations.

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3.07.P-Mo241 The Capability of Silicone Wristbands for Targeted and Suspect Screening of Toxic Compounds in Primary Schools

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Silicone wristbands (SWBs) have proven effective in assessing human exposure to environmental chemicals, while their chemical uptake capacity is still under investigation. Limited studies have examined SWB chemical uptake in controlled conditions, but variations in temperature, ventilation, and multiple pollution sources in real-world indoor settings may affect their performance. This study investigated the chemical uptake capacity of SWBs under varying temperatures, focusing on their potential as tools for

screening toxic chemicals in primary school classrooms where children spend significant time. Five classrooms were equipped with SWBs and polyurethane foam (PUF) passive samplers to monitor polycyclic aromatic hydrocarbons (PAHs), organophosphate esters (OPEs), and phthalate esters (PEs) in indoor air for 36 days. The sampling was repeated twice more at different air temperatures. All samples were screened for targeted and suspect compounds using GC-MS. As a result, phenanthrene, fluorene, and fluoranthene were the dominant PAHs, with decreasing concentrations as the temperature increased. Tri-*n*-butyl phosphate and tris(2-chloroethyl) phosphate were the abundant OPEs in the indoor air, and diethyl phthalate, diisobutyl phthalate, and di-*n*-butyl phthalate were the dominant PEs. With increasing temperatures, OPE and PE concentrations increased. The chemical uptake capacity of SWBs for target compounds generally decreased with the increased air temperature, although no statistical relationship was found. Suspect screening of toxic compounds in indoor air using SWBs and PUFs revealed 4-hydroxy-4-methylpentan-2-one, 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate, galaxolide, and Cyclopenta[*g*]-2-benzopyran as the priority compounds that have higher toxicity and abundance in indoor air. These chemicals, used mainly in surfactants, personal care products, cleaning products, and insect repellents, require regular monitoring and special efforts for reduction. The results demonstrated that SWBs can be used as passive air samplers to screen targeted and suspect compounds in indoor air.

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3.07.P-Mo242 Reaching High Throughput for Chemical Exposomics of Human Serum by Phospholipid Removal and LC – HRMS

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There is a growing interest to delve into the chemical exposome in human samples to help understand the potential link between diseases and our environment, lifestyle, and occupation. Blood chemical exposomics is facing a number of challenges to investigate sufficiently large-scale population cohorts, which typically provide hundreds to thousands of samples with a limited volume of serum/plasma. For instance, current methods investigating the blood chemical exposome are usually limited to a small number of target analytes with low throughput which does not support large cohort designs.

To overcome these issues, we optimized a protein precipitation and phospholipid removal method for sensitive chemical exposomics analyses in blood previously published by Sdougkou et al. (2023), and modified it to enable high throughput applications in 96-well plates with low sample volumes. Following protein and phospholipid removal of 100 µL serum, a partial evaporation step was optimized to improve the chromatographic separation and detection of 273 analytes, some of which are detected in both ionization modes, by liquid chromatography (LC) and high-resolution mass spectrometry (HRMS).

The targets that were selected for the method validation cover a wide variety of class uses (e.g., industrial, pharmaceuticals, pesticides, etc.), including endogenous metabolites (e.g., testosterone, progesterone, corticosterone). These targets are exhibiting a wide range of chemical properties, and are eluting between 5% (initial condition) and 100% of methanol using a gradient elution, with the earliest and latest eluting compound being trifluoromethanesulfonic acid (PFAS, eluting 0.5 min after the void volume) and 4-nonylphenol (various industrial uses), respectively.

The method proved to be repeatable, with 145 out of 155 targets detected in positive mode (93.5%) and 162 out of 168 targets detected in negative mode (96.4%) having a relative standard deviation (RSD) below 20%. Satisfactory recoveries (80-120%) were obtained for 109 of the targets (70.3%) in positive mode and 108 (64.3%) targets in negative.

Overall, this method holds promise to reach the high throughput needed for the comprehensive characterization of the human blood chemical exposome.

3.07.P-Mo243 Integrated Exposure Assessment of Bisphenol A and Bisphenol S Considering both Life Stages and Exposure Route

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Bisphenol A (BPA) and Bisphenol S (BPS) are endocrine-disrupting chemicals widely found in plastics, canned foods, dust, and thermal paper receipts. BPA is frequently detected in polycarbonate plastics and

epoxy resins, while the use of BPS as an alternative to BPA has increased in recent years. This study aimed to evaluate exposure levels of BPA and BPS and assess associated risks across different life stages. A literature review covering the years 2010 to 2023 was conducted to identify sources and concentrations of BPA and BPS in food, air, dust, and consumer products. A total of 92 studies on BPA and 32 studies on BPS were analyzed. In addition, eight capsule coffee samples were analyzed to detect BPA and BPS using liquid chromatography-mass spectrometry. Using data from the literature review and capsule coffee analysis, exposure amounts for each route were calculated using exposure scenarios. Exposure assessments for BPA and BPS were conducted across different life stages, including infants, children, adolescents, and adults. Regardless of life stages, grains showed the highest intake contribution rate among foods, followed by baby food (including breast milk and powdered milk) for infants and meat for adults. Infants and young children have a specific food consumption behavior, so when exposure targets were classified by life stages, differences were found in the main exposure sources. The results highlight the need for further monitoring of bisphenol analogues, particularly BPS, and their emerging sources. This study provides an integrated exposure assessment that can inform risk evaluations and regulatory decisions.

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3.07.P-Mo244 Innovative Non-Targeted Screening Approach using High-Resolution Mass Spectrometry for the Screening of Organic Chemicals and Identification of Specific Tracers of Soil and Dust Exposure in Children

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Environmental contamination through direct contact, ingestion and inhalation are common routes of children's exposure to chemicals, in which through indoor and outdoor activities associated with common hand-to-mouth, touching objects, and behavioral tendencies, children can be susceptible and vulnerable to organic contaminants in the environment. In this study, we have implemented and optimized a methodology based on non-targeted analysis (NTA) for comprehensive screening and identification of a wide range of organic contaminants in indoor dust, soil, food, drinking water, and urine matrices (N = 439) in the greater Miami area, Florida, U.S., prioritizing chemicals to assess children's environmental exposure. Families with children between 6 months and 6 years of age of underrepresented groups were recruited and samples were provided by the caregivers. The main goal was the selection of unique tracers of soil and dust ingestion in young children by NTA using Q-Exactive Orbitrap MS followed data processing by the Compound Discoverer software (v3.3, SP2). Identified features were plotted using Kendrick mass defect plot and Van Krevelen diagrams to show unique patterns in different samples and regions of anthropogenic compound classifications. Chemical features were first prioritized based on their predominant abundance (peak area > 500,000), detection frequency (in > 50% of the samples), available information on their uses and potential toxicological effects. Specific tracers of soil and dust exposure in children were selected in this study including Tripropyl citrate and 4-Dodecylbenzenesulfonic acid. The criteria for selection of the tracers were based on their higher abundance, detection frequency, unique functional uses, measurable amounts in urine (suitable biomarker), and with information on gastrointestinal absorption, metabolism, and excretion, and were further confirmed by authentic standards. We are proposing for the first time suitable unique tracers for dust ingestion by children.

3.07.P-Mo245 Deepen the Characterisation of the Perinatal Chemical Exposome Thanks to SS/NTS Global Profiling Strategies

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The exposome encompasses all environmental and socio-economic factors encountered over a lifetime, complementing the genome in explaining their roles in health outcomes. Since the concept's introduction in 2005, significant progress has been made in advancing methods, tools, and knowledge to assess the exposome and its impact on human health. However, chemical exposure during the perinatal period remains understudied, largely due to the challenges of accessing appropriate and sufficient amount of biological samples required to conduct multiple analysis to detect a broad range of chemicals.

To overcome these limitations, the application of innovative suspect screening (SS) and non-targeted screening (NTS) approaches based on high-resolution mass spectrometry (HRMS) offers a more effective approach by one analysis to characterize and chemically profile a wider range of substances. These advanced analytical approaches allow, with a single analysis, for the detection of a wide array of compounds covering a broad spectrum of physicochemical properties. This strategy not only enhances global human biomonitoring but also improves the identification of chemicals of emerging concern in an early warning perspective.

We have developed streamlined workflows, from sample preparation to data analysis, to investigate various maternal (urine, serum, maternal milk) and foetal (cord blood, amniotic fluid) biological matrices from diverse mother-child cohorts. These methods were applied in a range of research projects conducted at national and EU level. The proposed presentation will describe these different proof-of-concepts enabling the determination of real-life chemical mixtures composing the internal exposure related to the perinatal period

3.08 Sampling and Analysis Methodologies, and Environmental Behavior Studies for Emerging Organic Contaminants in the Aquatic Environment: Recent Advances and Perspectives

3.08.T-01 Passive Sampling of PFAS in the Aquatic Environment

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Per- and polyfluoroalkyl substances (PFAS) are a group of compounds of high concern due to their ubiquity, persistence, and adverse health impacts. With a diversity of chemical structures and properties, versatile detection tools are needed to capture as many PFAS as possible. Different passive samplers (microporous polyethylene tube, MPT, and a diffusive gradients in thin film, DGT, were hence calibrated for the uptake of different PFAS. The MPT was tested with target PFAS, and suspect PFAS and extractable organofluorine (EOF) during 1-2 week deployments in groundwater, a freshwater river, and an estuary contaminated by aqueous film-forming foam (AFFF). Targeted analysis, suspect screening, and EOF were performed on passive and grab samples to derive sampling rates, R_s . Median measured and fluorine-normalized estimated EOF R_s in groundwater (7.1 vs 8.4 mL day⁻¹ respectively) and river water (55 vs 66 mL day⁻¹ respectively) were within 20% of each other. For suspect PFAS, R_s were similar to targeted PFAS of alike functional group chemistry and perfluorinated carbon chain length. For example, for 6:2 and 8:2 FTSAS-sulfoxide, estimated R_s were 1.8- and 6.0-mL day⁻¹, respectively, similar to R_s measured for 6:2 and 8:2 FTS of 2.2- and 6.3-mL day⁻¹. These results suggest targeted and suspect PFAS, and EOF, are taken up by MPT passive samplers in a predictable manner, expanding passive sampling capabilities. The DGT sampler, in contrast, we calibrated mostly to test the effect of temperature on sampling rates based on a diffusive model. In joint deployments, results from the DGT were closer to grab sample results, while the PMT was sturdier during field deployments, with greater recovery rates. Both samplers offer the advantage of being able to derive time-weighted average concentrations of PFAS in the field, with the potential to estimate those for non-target and suspect PFAS, too.

3.08.T-02 Evaluation of the Performances of a Biofilm-Based Passive Sampler to Monitor Micropollutants in Wastewater

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Passive samplers (PS) are powerful tools for monitoring micropollutants due to their ability to accumulate them over weeks or months, providing great time-representativeness. Additionally, they enable to achieve lower detection limits compared to traditional methods like grab or composite sampling.

However, in wastewater this sampling method faces the constraint of biofouling, which hampers the transfer of micropollutants from water to the receiving phase. This work aims to investigate an innovative PS, the Prebio cell STEP, that uses the biofilm as a receiving phase, and thus turns biofouling into an advantage.

The operating conditions of the Prebio cell STEP were defined, through the kinetic study of biofilm growth, and accumulation of targeted micropollutants over several months in influents and effluents of an urban wastewater treatment plant (WWTP), to determine the optimal deployment duration of this sampler. In parallel, the performances of the Prebio cell STEP were compared with conventional 24h water composite sampling regarding the detection of the same micropollutants targeted for the kinetic study, and number of compounds detected by suspect and non-target screenings using LC/HRMS analysis. First results showed major differences in appearance of biofilm, and growth kinetics between WWTP

influent and effluent which could be explained in relation with the various complexity of these water matrices.

Chemical analysis of the biofilm revealed the ability of the biofilm to capture micropollutants present in the wastewater samples. The kinetic of accumulation of some of these micropollutants in the biofilm will be presented (metals, alkylphenols, polycyclic aromatic hydrocarbons). Moreover, LC/HRMS suspect and non-target screening highlighted the presence of a variety of micropollutants in the biofilm, including some not detected in wastewater composite samples.

3.08.T-03 Pesticide Pathways Revealed: Comprehensive Analytical Methods for Multi-Compound Monitoring in Urban and Agricultural Catchments

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Pesticides can pose an ecotoxicological risk to humans and aquatic organisms. To develop effective mitigation measures, it is essential to investigate their environmental entry pathways. However, their authorisation for different applications (i.e., as plant protection products or as biocides) makes source identification challenging. Moreover, nonpolar pesticides, such as pyrethroids, are toxic at low pg/L concentrations, tend to adsorb to particles and may bioaccumulate due to their hydrophobicity. Combining sensitive analytical methods is indispensable to determine the total concentration (i.e., the dissolved and the particle-bound fractions) and the concentration in the dissolved fraction only. This study aims to a) assess the occurrences of a broad spectrum of pesticides and b) gain insights on their entry pathways from urban and agricultural catchments.

River and wastewater effluent samples were collected from February to November 2023 at five different sites. A liquid-liquid extraction followed by extract splitting and analysis using gas chromatography with atmospheric pressure chemical ionisation (GC-APCI) or liquid chromatography with electrospray ionisation (LC-ESI) coupled to a triple quadrupole mass spectrometer (QqQ-MS) enabled the quantification of the total concentration of 70 nonpolar compounds ($\text{LogP} > 4$). For the quantification of about 240 more polar pesticides in the dissolved fraction, enrichment by vacuum-assisted evaporative concentration followed by LC-ESI coupled to a high-resolution mass spectrometer (HRMS) was used. Glyphosate was measured using ion chromatography HRMS.

Pyrethroids - namely lambda-cyhalothrin, cypermethrin, permethrin, deltamethrin - remain the main risk drivers, with contributions from previously understudied nonpolar pesticides (e.g., fipronil, acetonitrile, diflufenican) and more polar insecticides (e.g., imidacloprid, thiacloprid) and herbicides (e.g., dimethachlor, metazachlor, nicosulfuron). Approximately 80% of the detected substances enter the environment largely via wastewater treatment plants. Hypotheses about the source can be made by considering the regulatory data and the mode of action of the substance in question, together with site-specific information like land use, meteorology, and hydrology. The present study offers important data on the main entry pathways of non- and polar pesticides, thereby providing critical insights for effective mitigation strategies.

3.08.T-04 Dynamics of Persistent and Mobile Chemicals, Including Ultra-Short Per- and Polyfluoroalkyl Substances, in Groundwater: Distribution, Influencing Factors, and Risk

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Persistent and mobile (PM) chemicals, including pesticides, pharmaceuticals, industrial compounds, and PFAS (per- and poly-fluoroalkyl substances), pose significant environmental and public health concerns due to their resistance to degradation and potential toxicity. These chemicals, along with their transformation products, are often challenging to detect in previous monitoring, resulting in gaps in understanding their occurrence, behavior, and risks. Our study investigates the distribution, influencing factors, and risks of PM chemicals in groundwater from Saxony, Germany. 82 groundwater samples from 25 groundwater bodies were collected and analysed for 180 PM chemical candidates using our optimized analytical method, including freeze-drying and supercritical fluid chromatography mass spectrometry measurement (capable of trace analysis). A total of 163 PM chemicals were detected with the median summed PM chemical concentration of 23,000 ng/L. Trifluoroacetic acid (TFA) was identified as the most prevalent compound, with concentrations reaching $\mu\text{g L}^{-1}$ levels. Statistical analysis revealed that PM chemical properties, such as solubility and soil-water partitioning, significantly influenced PM chemical distribution, while lipophilicity ($\log D$) showed limited correlation. Water characteristics, including inorganic ions (e.g., nitrate, sulfate, calcium, and sodium), also played a pivotal role in shaping PM chemicals distribution. Cluster analysis highlighted co-occurrence patterns, identifying benzothiazole and N,N-dimethylformamide as key indicators of mixed contamination from industrial sources. Spatial

distribution patterns linked PM chemicals to regional land-use activities, with certain compounds as potential indicators of specific pollution sources. Risk assessment using ToxPi (Toxicological Priority Index) scoring and risk indices prioritized 35 PM chemicals with the highest environmental and public health risks, including TFA, benzothiazole, N-methylpiperidine, and chloridazon-desphenyl, suggesting that agricultural and industrial activities are the major sources for the groundwater bodies as at higher risk levels. These findings provide insights into the behaviors of PM chemicals in groundwater and suggest the need of tailored monitoring, regulation, and remediation strategies to mitigate PM chemical risks.

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3.08.P-Th116 Source Control of Industrial Emissions: Monitoring Strategies to Discern Pollutant Spills

Tom Galle¹, Denis Pittois¹, Viola Huck¹, Carole Lacroix², Laurene Chochois², Parviel Chirsir³, Emma Schymanski⁴, Kenzo Fabbro⁵, Carine Guilloteaux⁵ and Raymond Erpelding⁵, (1)Erin, Luxembourg Institute of Science and Technology (LIST), Luxembourg, (2)Luxembourg Institute of Science and Technology (LIST), Luxembourg, (3)University of Luxembourg, Luxembourg, (4)Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Luxembourg, (5)SIACH, Luxembourg

Micropollutant emissions from industry often blend with household contributions stemming from consumer goods. In addition, industrial emissions can be very sporadic. There are therefore two major challenges that need to be addressed to assess the emissions in the technical catchment of a WWTP: covering a large enough period of time to catch emissions with low return times and identifying the compounds that are emitted. The SourceControl project adopted a monitoring strategy that combined collecting samples at the inlet of a WWTP with passive and autosamplers with non-target analysis of soluble compounds (LC-MS). Passive samplers were deployed for one week each during 8 weeks in total while autosamplers covered daily dynamics (3-hour mixed samples) over three weeks within that period. Passive sampler extracts were first screened with non-target analysis to identify compounds of relevance and then quantified with target analysis. The combination of statistical analysis of autosampler results and in sewer tracking allowed to dress emitter profiles. WWTP inlets show typical diurnal dynamics of domestic organic loads in highly resolved autosampler campaigns. NH₄ and DOC were used to verify the regularity of household emissions and the absence of strong industrial contributors. Pharmaceuticals and in-house biocides were strongly correlated to NH₄ and DOC. Pollutants with diffusive emission from consumer goods like flame retardants had a binary behaviour with a background emission from households and peak spills from industry (example TCPP). Some compounds showed very regular emission patterns from industry and were associated to other compounds showing synchronized peaks. A semi-quantitative screening with passive-samplers in sewer systems at points in the network that isolated distinct industrial zones allowed for specific source allocation. Two single weeks were covered by passive samplers and the stronger precipitation during one week enabled a cluster analysis to discern specific industrial inputs but also continuous domestic input from urban surface runoff. The approach complemented the results of the WWTP inlet analysis revealing similar pollutant profiles. The contribution will discuss the opportunities and limits of combining different monitoring and analytical methods as well as the further investigations needed for the identified compounds.

Disclaimer/Disclosure: The project SourceControl was granted by the Luxembourgish Water Management Fund.

3.08.P-Th117 Wide Pesticide Screening Assessment in Aquatic Environments: Comparisons of Sampling and Extraction Techniques

Caterina Cacciatore¹, Jackie Myers¹, Giulio Mariani², Bernd Manfred Gawlik² and Vincent J. Pettigrove³, (1)RMIT University, Australia, (2)European Commission, Joint Research Centre (JRC), Italy, (3)Aquest Research Group, RMIT University, Australia

Amidst increasing use of pesticides worldwide and their discharge into aquatic environments, there is a need improve our understanding of pesticide occurrence and improve water quality monitoring. Addressing the data gaps associated with pesticide contamination, this research combines wide-screening technology and citizen engagement. By employing both passive and grab sampling methods alongside wide-screening techniques, we can detect a broader range of pesticides in water. Involving local citizen groups in water quality monitoring expands the temporal and spatial scale of environmental data. This strategic collaboration between society and scientists not only strengthens community connection but also

raises awareness about sustainable water quality management.

In the context of sampling and analytical techniques for wide pesticide screening, this research examines the synergies, advantages and disadvantages of passive sampling (Chemcatcher and POCIS) and grab sampling in combination with Solid Phase Extraction (SPE), and the Stir Bar Sorptive Extraction (SBSE). The comparison encompasses the number and concentration of detected pesticides, based on a wide-screen of 230 compounds analysed by Gas Chromatography Quadruple Time of Flight High Resolution Mass Spectrometry. The evaluation of the analytical techniques is grounded in field data from a month-long monitoring campaign conducted in March 2023 at three sampling sites in Melbourne, Australia. Grab sampling coupled with SBSE detected the highest number of pesticides, though its quantification was 2 to 3 times lower than that of grab samples extracted by SPE. Passive samplers detected the fewest pesticides. The hydrophobicity and characteristics of the sampling locations partly explained these results. The advantages and disadvantages of the different sampling and extraction methods have been analysed in terms of temporal information provided, sensitivity, complexity of the analytical procedures, and their applicability for citizen-based water quality monitoring. Beyond the novelty of evaluating different analytical techniques for an extensive list of pesticides, this study is significant due to its implementation in a citizen engagement initiative by the Joint Research Centre of the European Commission. This presentation will highlight the comparative effectiveness of the different wide-screening techniques and explores the potential of citizen science to contribute to monitoring pesticides in water.

3.08.P-Th120 Occurrence, Remove Efficiency and Modelling of Pharmaceutical and Personal Care Products (PPCPs) in a Typical Wastewater Treatment Plant in Wuhan

Zhe Qian¹, Chi Zhang², Shihua Qi³ and Wei Chen², (1)State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, China (Mainland), (2)School of Environmental Studies, and MOE Key Laboratory of Groundwater Quality and Health, China University of Geosciences, China (Mainland), (3)State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences; School of Environmental Studies, and MOE Key Laboratory of Groundwater Quality and Health, China University of Geosciences, China (Mainland)

Pharmaceuticals and personal care products (PPCPs) are among the emerging organic contaminants, posing significant risks to human health and ecological safety. Wastewater treatment plants (WWTPs) serve as critical control points for the release of PPCPs into the environment. However, the high data uncertainty in current research compromises the accuracy of exposure model predictions. In this study, the occurrence, temporal variation and removal efficiency of 15 PPCPs across 5 categories were investigated in a WWTP using diffusive gradients in thin-films (DGT) technology. The EU exposure model SimpleTreat 4.0 was modified and optimized according to the characteristics of the Chinese WWTP scenario. Accurate data obtained through DGT were used to validate the model predictions and assess their reliability. A total of 11 PPCPs were detected in influent and effluent, with total concentrations ranging from 141-5287 ng L⁻¹. The time-averaged weighted concentrations provided by passive sampling effectively reduced data uncertainty. Temporal variations in preservatives, estrogens, antimicrobials, and antioxidants in influent primarily related to changes in rainfall and temperature, while bisphenols were likely impacted by industrial production activities. Additionally, changes in PPCP concentrations may also have been influenced by the effects of the COVID-19 pandemic. PPCPs were mainly removed in the secondary treatment. Among the 31 basic WWTP scenario parameters in the SimpleTreat 4.0 model, sensitivity analysis identified 11 parameters with a significant impact on model predictions, and 7 of which were optimized. The improved model yielded more accurate prediction for bisphenols, antioxidants and biocides.

Disclaimer/Disclosure: The authors thank financial supporting by the National Natural Science Foundation of China (41907327 and 42007178), the Natural Science Foundation of Hubei (2019CFB372), the National Key Research and Development Program (2019YFC1805502), and the Fundamental Research Funds (G1323523063) for the Central Universities, China University of Geosciences (Wuhan), and the assistance from the staff from the WWTPs for the sample collection.

3.08.P Sampling and Analysis Methodologies, and Environmental Behavior Studies for Emerging Organic Contaminants in the Aquatic Environment: Recent Advances and Perspectives

3.08.P-Th115 Tracking Pesticide Peaks: Time-Integrated vs. Flow-Proportional Sampling in Agricultural Streams

Kajsa Weslien, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

A variety of factors affect the leaching of pesticides from treated agricultural land. For instance, heavy

rainfall can transport pesticides into nearby streams, generating water flow peaks where pesticide concentrations can be temporarily elevated. How and when surface water samples are collected will consequently have a large impact on the results. Conventional water quality monitoring based on grab sampling, typically done once or every three months, can struggle to provide reliable estimates when sampling streams due to the temporal variability in pesticide concentration.

In this study, we compare two sampling methods used within the Swedish national pesticide monitoring program, time-integrated and flow-proportional sampling, focusing on their ability to capture temporal peak concentrations linked to high flow events. In time-integrated sampling, 100 subsamples are collected each week to give weekly average concentrations of each substance, reflecting the long-term, chronic exposure of pesticides in the stream. In contrast, flow-proportional sampling involves collecting samples in proportion to stream flow velocity, with more samples collected during higher flow conditions, capturing short-term, acute toxicity. The monitoring program targets four small agricultural catchments in southern Sweden, with flow-proportional and time-integrated sampling used simultaneously in one catchment since 2009. Data from 2009-2021 are included in the comparisons.

Our results show that pesticide concentrations in small streams can vary more than hundredfold within just a few hours during high flow events. Flow-proportional samples collected during these events generally display higher pesticide concentrations than weekly time-integrated samples collected over the same time period, often reaching concentrations around ten times higher than the weekly average. Flow-proportional sampling can therefore provide valuable insights into the large variability of pesticide concentrations in agricultural streams, which is important as even short-term peaks in pesticide concentrations (hours to days) can have negative effects the ecosystem. Together, the two sampling methods offer a comprehensive understanding of both the chronic and acute impacts of pesticides in streams, insights that can be overlooked when relying solely on grab sampling.

3.08.P-Th116 Source Control of Industrial Emissions: Monitoring Strategies to Discern Pollutant Spills

Tom Galle¹, Denis Pittois¹, Viola Huck¹, Carole Lacroix², Laurene Chochois², Parviel Chirsir³, Emma Schymanski⁴, Kenzo Fabbro⁵, Carine Guilloteaux⁵ and Raymond Erpelding⁵, (1)Erin, Luxembourg Institute of Science and Technology (LIST), Luxembourg, (2)Luxembourg Institute of Science and Technology (LIST), Luxembourg, (3)University of Luxembourg, Luxembourg, (4)Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Luxembourg, (5)SIACH, Luxembourg

Micropollutant emissions from industry often blend with household contributions stemming from consumer goods. In addition, industrial emissions can be very sporadic. There are therefore two major challenges that need to be addressed to assess the emissions in the technical catchment of a WWTP: covering a large enough period of time to catch emissions with low return times and identifying the compounds that are emitted. The SourceControl project adopted a monitoring strategy that combined collecting samples at the inlet of a WWTP with passive and autosamplers with non-target analysis of soluble compounds (LC-MS). Passive samplers were deployed for one week each during 8 weeks in total while autosamplers covered daily dynamics (3-hour mixed samples) over three weeks within that period. Passive sampler extracts were first screened with non-target analysis to identify compounds of relevance and then quantified with target analysis. The combination of statistical analysis of autosampler results and in sewer tracking allowed to dress emitter profiles. WWTP inlets show typical diurnal dynamics of domestic organic loads in highly resolved autosampler campaigns. NH₄ and DOC were used to verify the regularity of household emissions and the absence of strong industrial contributors. Pharmaceuticals and in-house biocides were strongly correlated to NH₄ and DOC. Pollutants with diffusive emission from consumer goods like flame retardants had a binary behaviour with a background emission from households and peak spills from industry (example TCPP). Some compounds showed very regular emission patterns from industry and were associated to other compounds showing synchronized peaks. A semi-quantitative screening with passive-samplers in sewer systems at points in the network that isolated distinct industrial zones allowed for specific source allocation. Two single weeks were covered by passive samplers and the stronger precipitation during one week enabled a cluster analysis to discern specific industrial inputs but also continuous domestic input from urban surface runoff. The approach complemented the results of the WWTP inlet analysis revealing similar pollutant profiles. The contribution will discuss the opportunities and limits of combining different monitoring and analytical methods as well as the further investigations needed for the identified compounds.

Disclaimer/Disclosure: The project SourceControl was granted by the Luxembourgish Water Management Fund.

3.08.P-Th117 Wide Pesticide Screening Assessment in Aquatic Environments: Comparisons of Sampling and Extraction Techniques

Caterina Cacciatori¹, Jackie Myers¹, Giulio Mariani², Bernd Manfred Gawlik² and Vincent J. Pettigrove³, (1)RMIT University, Australia, (2)European Commission, Joint Research Centre (JRC), Italy, (3)Aquest Research Group, RMIT University, Australia

Amidst increasing use of pesticides worldwide and their discharge into aquatic environments, there is a need to improve our understanding of pesticide occurrence and improve water quality monitoring.

Addressing the data gaps associated with pesticide contamination, this research combines wide-screening technology and citizen engagement. By employing both passive and grab sampling methods alongside wide-screening techniques, we can detect a broader range of pesticides in water. Involving local citizen groups in water quality monitoring expands the temporal and spatial scale of environmental data. This strategic collaboration between society and scientists not only strengthens community connection but also raises awareness about sustainable water quality management.

In the context of sampling and analytical techniques for wide pesticide screening, this research examines the synergies, advantages and disadvantages of passive sampling (Chemcatcher and POCIS) and grab sampling in combination with Solid Phase Extraction (SPE), and the Stir Bar Sorptive Extraction (SBSE). The comparison encompasses the number and concentration of detected pesticides, based on a wide-screen of 230 compounds analysed by Gas Chromatography Quadrupole Time of Flight High Resolution Mass Spectrometry. The evaluation of the analytical techniques is grounded in field data from a month-long monitoring campaign conducted in March 2023 at three sampling sites in Melbourne, Australia. Grab sampling coupled with SBSE detected the highest number of pesticides, though its quantification was 2 to 3 times lower than that of grab samples extracted by SPE. Passive samplers detected the fewest pesticides. The hydrophobicity and characteristics of the sampling locations partly explained these results. The advantages and disadvantages of the different sampling and extraction methods have been analysed in terms of temporal information provided, sensitivity, complexity of the analytical procedures, and their applicability for citizen-based water quality monitoring. Beyond the novelty of evaluating different analytical techniques for an extensive list of pesticides, this study is significant due to its implementation in a citizen engagement initiative by the Joint Research Centre of the European Commission.

This presentation will highlight the comparative effectiveness of the different wide-screening techniques and explores the potential of citizen science to contribute to monitoring pesticides in water.

3.08.P-Th118 Analysis of Pesticides, Pharmaceuticals and Personal Care Products in Water Samples According to the Proposal for a Directive Amending the EU Water Framework Directive

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The monitoring of harmful substances in drinking and environmental water is essential for protecting human health and maintaining environmental quality standards. Accurate measurements at ultra-low levels are a crucial part of any trace monitoring program. As more pesticides, pharmaceuticals, and personal care products (PPPCs) are released into the environment, there is a growing demand for analytical methods that can handle multiple compound groups. One major analytical challenge lies in the wide chemical diversity of compound classes and structures. Annex V of the proposal for a Directive amending the Water Framework Directive lists Environmental Quality Standards (EQS) for priority substances in surface water. This list of substances was extended to include more pesticides, pharmaceuticals, and personal care products, forming the basis of the presented work. Further compounds of interest were added based on our experience with requests from water testing labs in Europe.

The purpose of this work was to demonstrate a direct injection UPLC-MS/MS method for the ultra-low-level determination of PPPCs in drinking and environmental waters, in alignment with the EU WFD proposal. The method performance study was completed on an ACQUITY Premier LC System with a Xevo TQ Absolute MS system. A method validation study was carried out on drinking and surface water matrices. Average method performance for trueness, repeatability, linearity, and sensitivity was assessed through inter- and intra-laboratory studies.

The sensitivity of the UPLC-MS/MS system allows for the use of a direct injection approach, which eliminates complex sample preparation and enables the simple, high-throughput analysis of pesticides, pharmaceuticals, and personal care products in drinking and environmental water.

3.08.P-Th119 Pharmaceutical and Personal Care Products (PPCPs) in Typical Wastewater Treatment Plants (WWTPs) in Wuhan, China

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(Mainland), (3)South China Institute of Environmental Sciences, Ministry of Ecology and Environment, China (Mainland), (4)Lancaster Environment Centre, Lancaster University, United Kingdom

Pharmaceuticals and personal care products (PPCPs) are a group of daily used compounds that may have adverse effects on organisms even at trace concentrations, so PPCPs have become one of the research hotspots in recent years. To Understand the occurrence and removal of PPCPs in wastewater treatment plants (WWTPs) is of great significance for the prevention and control of PPCPs. This study investigated nine WWTPs with different processes in Wuhan and used high performance liquid chromatography tandem-mass spectrometry (HPLC-MS/MS) to study the occurrence and distribution of 75 PPCPs in three media (dissolved phase, suspended particle phase, and sludge) in the WWTPs. The main conclusions are as follows:

The concentration ranges in the influent dissolved phases, effluent dissolved phases, influent suspended particle phases, effluent suspended particle phases and sludges from the 9 WWTPs were 666 - 3585 ng·L⁻¹, 103 - 1287 ng·L⁻¹, 196 - 8473 ng·L⁻¹, 4.51 - 130 ng·L⁻¹ and 2479 - 14224 ng·g⁻¹ (dry weight), respectively. Antibiotics were the PPCPs with the highest concentrations, followed by nonsteroidal anti-inflammatory drugs and β -blockers. The distribution patterns of PPCPs in the three media were related to the physicochemical properties of the target substances, with hydrophobic PPCPs having higher concentrations in the suspended particle phase or sludge.

The removal efficiencies of different PPCPs in different WWTPs showed that the nine WWTPs in Wuhan had relatively high removal efficiencies for target substances, with an overall average removal efficiency of 70.8%. Biological treatment is the main unit for removing PPCPs, which is mainly achieved through microbial degradation and sludge adsorption. Different biological treatment processes have different removal efficiencies on target PPCPs, with the anaerobic-anoxic-aerobic and membrane bioreactor processes having higher removal efficiencies than the oxidation ditch and sequencing batch reactor processes.

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3.08.P-Th120 Occurrence, Remove Efficiency and Modelling of Pharmaceutical and Personal Care Products (PPCPs) in a Typical Wastewater Treatment Plant in Wuhan

Zhe Qian¹, Chi Zhang², Shihua Qi³ and Wei Chen², (1)State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, China (Mainland), (2)School of Environmental Studies, and MOE Key Laboratory of Groundwater Quality and Health, China University of Geosciences, China (Mainland), (3)State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences; School of Environmental Studies, and MOE Key Laboratory of Groundwater Quality and Health, China University of Geosciences, China (Mainland)

Pharmaceuticals and personal care products (PPCPs) are among the emerging organic contaminants, posing significant risks to human health and ecological safety. Wastewater treatment plants (WWTPs) serve as critical control points for the release of PPCPs into the environment. However, the high data uncertainty in current research compromises the accuracy of exposure model predictions. In this study, the occurrence, temporal variation and removal efficiency of 15 PPCPs across 5 categories were investigated in a WWTP using diffusive gradients in thin-films (DGT) technology. The EU exposure model SimpleTreat 4.0 was modified and optimized according to the characteristics of the Chinese WWTP scenario. Accurate data obtained through DGT were used to validate the model predictions and assess their reliability. A total of 11 PPCPs were detected in influent and effluent, with total concentrations ranging from 141-5287 ng L⁻¹. The time-averaged weighted concentrations provided by passive sampling effectively reduced data uncertainty. Temporal variations in preservatives, estrogens, antimicrobials, and antioxidants in influent primarily related to changes in rainfall and temperature, while bisphenols were likely impacted by industrial production activities. Additionally, changes in PPCP concentrations may also have been influenced by the effects of the COVID-19 pandemic. PPCPs were mainly removed in the secondary treatment. Among the 31 basic WWTP scenario parameters in the SimpleTreat 4.0 model, sensitivity analysis identified 11 parameters with a significant impact on model predictions, and 7 of which were optimized. The improved model yielded more accurate prediction for bisphenols, antioxidants and biocides.

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the National Key Research and Development Program (2019YFC1805502), and the Fundamental Research Funds (G1323523063) for the Central Universities, China University of Geosciences (Wuhan), and the assistance from the staff from the WWTPs for the sample collection.

3.08.P-Th121 Seasonal Study of the Presence of Pharmaceuticals in Mero-Barcés River Basin (NW Spain)

Jorge Lejo Santiago, Estefania Concha-Graña, Purificación Lopez-Mahia and Soledad Muniategui-Lorenzo, University of A Coruña, Spain

Pharmaceuticals are being found in the environment because WWTPs do not have the technology to remove all the organic pollutants. These compounds reach WWTPs through wastes or excretions by human and animal after their consumption. Due to this situation, the EU has recently approved the Urban Waste Water Treatment Directive (91/271/EEC) which will regulate 11 pharmaceuticals. As it will be a challenge to remove the different CECs, it is important to have environmental data on which compounds affect the ecosystem and need to be removed.

The aim of this work is to study the presence and behavior of 56 pharmaceuticals in a reservoir where drinking water is provided to the population using liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS (QqQ)) for their determination. The seasonal study was performed collecting samples from 7 sampling sites in the Mero-Barcés River Basin (NW Spain). These points represent the whole river basin taking points in the two rivers before they reach the reservoir, two points inside the reservoir where the water of both rivers is not yet mixed, one point where the water is mixed, another in the dam and the last one in the river after leaving the reservoir. Each point was measured during 6 seasons between June 2022 and December 2023.

37 pharmaceutical compounds were detected at levels ranging from 0.1 to 143 ng/L, with antibiotics, cardiovascular drugs, NSAIDs and psychiatric drugs being the most representative therapeutic classes. Of all the pharmaceuticals found in the samples, 5 of them are proposed to be regulated by the EU in Surface (azithromycin, carbamazepine, clarithromycin, diclofenac and erythromycin). There appears to be an increase in the concentrations of pharmaceuticals during summer and fall, which could be due to the rainfall during these seasons. All therapeutic classes studied were found distributed in all sampling points except for anticoagulants, which were found in only 3 of the 7 sites.

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3.08.P-Th122 Seasonal Trends and Consumption Habits of Addictive Substances in the Community of Madrid (Spain) Through Wastewater Analysis

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The consumption of illegal drugs, as well as benzodiazepines, whether prescribed or not, has increased considerably in recent years in Spain. Their continued consumption leads to addiction, becoming a problem that harms both the consumer and society. Therefore, accurate and updated data on their use is essential.

In this work, wastewater-based epidemiology was applied to expand the existing information on the consumption habits of the population of the Community of Madrid (Spain). A study was carried out on the consumption of about twenty addictive substances, including illegal drugs, new addictive substances and benzodiazepines. The samples were collected from eight wastewater treatment plants (WWTP) at four different times: December 2023 (winter), April 2024 (spring), June 2024 (summer) and October 2024 (autumn) (224 samples in total). This made it possible to assess whether consumption of the investigated substances varied among the populations investigated and whether it changed according to the time of the year. Samples were analyzed using an optimized analytical method based on solid phase extraction (SPE) followed by liquid chromatography-coupled to mass spectrometry (HPLC-MS/MS), using a triple quadrupole analyzer.

Our data reflect that, in the Community of Madrid, cannabis and cocaine are by far the most consumed illegal substances. Among the new psychoactive substances (NPS) investigated, ketamine was found in all the samples whereas other NPS, such as mephedrone, were measured in a few samples. In the case of benzodiazepines, two of them (lorazepam and lormetazepam) were present in all the samples analyzed.

Regarding the differences in consumption observed between the populations investigated, statistical analysis indicated that they are not due to sociodemographic or socioeconomic factors but rather to the consumption habits of each population. On the other hand, similar consumption levels were observed throughout the year, with no clear seasonal variation, except for certain substances like codeine, whose consumption, in some populations, was slightly higher in December 2023; this variation could be attributed to its legal use in medications to relieve symptoms of cough and colds, which are more common in winter.

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3.08.P-Th123 Nationwide Monitoring of Organophosphate Esters in Korean Surface Waters

Junseong Park¹, Sori Mok¹, Daeho Kang², Jun Yub Kim³, Sun-Hong Lee⁴, Sojeong Han⁴, Tae Jin Park⁴, Sang Don Kim⁵, Junho Jeon² and Hyo-Bang Moon¹, (1)Department of Marine Science and Convergence Technology, College of Science and Convergence Technology, Hanyang University, Republic of Korea, (2)Department of Environmental Engineering, Changwon National University, Republic of Korea, (3)Water Environmental Management Dept., Korea Water Resources Corporation (K-water), Republic of Korea, (4)Water Environmental Engineering Research Division, National Institute of Environmental Research, Environmental Research Complex, Republic of Korea, (5)School of Earth Sciences and Environmental Engineering, Gwangju Institute of Science and Technology, Republic of Korea

With the regulation of production and use of halogenated flame retardants, their alternative, organophosphate esters (OPEs), are considered emerging concerns due to severe contamination in aquatic environment. OPEs are widely applied as flame retardant and plasticizer in a wide range of commercial and industrial products, such as electronic equipment, furniture, rubber, lubricant, and textile. In this study, nationwide monitoring of OPEs in Korean surface water was conducted to elucidate their occurrences and profiles, providing essential data for effective management. Surface water samples were collected from 130 sites of four major rivers of Korea the Han, Nakdong, Geum, and Yeongsan Rivers during three sampling campaigns in 2023. Gas chromatography and liquid chromatography with high-resolution mass spectrometry (GC-HRMS and LC-HRMS) were employed to identify and quantify 18 target OPEs. Tris(1-chloro-2-propyl) phosphate (TCIPP) was predominant compound in all river water samples, accounting for over 50% of the total OPEs, followed by tris(2-butoxyethyl) phosphate (TBOEP) and tris(2-chloroethyl) phosphate (TCEP). Generally, the Han River exhibited higher concentrations of OPEs compared to the other rivers, likely due to the proximity to urbanized areas. Relatively high concentrations of OPEs were observed near the outlets of wastewater and sewage treatment plants, implying that these facilities serve as point sources of OPE contamination. Several sites exhibited distinct contamination patterns, indicating the need for detailed investigations to identify the sources of contamination. Seasonal variation was observed in TCIPP, with relatively lower concentrations during the summer; however, no significant differences were found in TBOEP and TCEP. This study provides fundamental data for developing effective chemical management strategies for OPEs in aquatic environments.

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3.08.P-Th124 Nationwide Evaluation of Polyfluoroalkyl Substances Abundance in Reservoirs of Taiwan

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Per- and polyfluoroalkyl substances (PFASs) are a group of versatile chemicals known for their exceptional properties and associated toxicity. These substances can enter and disperse throughout the environment through various pathways. Concerns about PFASs have been growing due to emerging

research highlighting their increased health risks and complex behaviors, particularly with newly identified PFAS compounds. Reservoirs in Taiwan are critical in providing water for domestic, agricultural, and industrial use, making it essential to maintain water quality and prevent harmful contaminants from impacting public health. To assess the potential sources and risks of 10 PFASs, water samples were collected from the surface and bottom layers of six reservoirs in Taiwan between November 2019 and April 2020. The concentrations of PFASs were measured using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC/MS-MS). The studied reservoirs included Feicui Reservoir, Shimen Reservoir, Liyutan Reservoir, Mingde Reservoir, Renyitan Reservoir, and Cheng Ching Lake Reservoir. The total concentrations of PFASs ranged from below the limit of detection (LOD) to 12.3 ng/L in the surface water and from below the LOD to 9.93 ng/L in the bottom water. The predominant compounds detected were perfluorooctane sulfonic acid (PFOS), perfluorobutane sulfonic acid (PFBS), and perfluorooctanoic acid (PFOA). Their average concentrations in the surface water were 1.09 ng/L, 0.86 ng/L, and 0.85 ng/L, respectively, while in the bottom water, the average concentrations were 0.92 ng/L, 1.06 ng/L, and 0.75 ng/L, respectively (Mann-Whitney U test, p -value > 0.05). Source apportionment was conducted using principal component analysis (PCA) and positive matrix factorization (PMF), identifying two primary sources. The first source was attributed to domestic daily activities and metal plating processes, while the second source was linked to food packaging, outdoor equipment, and consumer products. Risk quotients (RQs) and the Health Risk Index (HRI) were calculated to evaluate potential risks. The RQ values were all below 0.01, indicating negligible risk to aquatic organisms. Similarly, HRI values were below 1, suggesting that consumption of treated drinking water from these reservoirs poses no significant health risks to the Taiwanese population.

3.08.P-Th125 Quantifying the Impact of Photolysis on Micropollutant Attenuation in a Natural River System

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Natural removal processes play a crucial role in the environmental fate of organic micropollutants, yet there remain considerable gaps in understanding the environmental factors that influence their relative importance and spatial and temporal variability. Micropollutants, such as pharmaceuticals and personal care products, are widely detected in water bodies and subject to a complex interplay of removal processes, including photolysis, adsorption, and biodegradation. While field studies have advanced the in-situ quantification of micropollutant attenuation in river systems, they often fail to decouple biodegradation widely regarded as the most significant mechanism from other simultaneous processes. Photolysis, in particular, presents a challenge due to its dependence on spatially and temporally variable factors such as water transparency, dissolved organic matter, and solar irradiance. Knowledge on the dynamics of the relative importance of photolysis with respect to overall removal of micropollutants in natural and urban streams is scarce but considered essential for predicting micropollutant behavior and informing water management strategies.

This study aims to distinguish micropollutants susceptible to photolysis under real-world conditions from those that are not. We conducted two consecutive field sampling campaigns, one at night and one during the day, under the same hydrological conditions along a 5 km stretch of the Ammer River near Tübingen, Germany. Using fluorescein dye tracer injections, we measured accurate travel times for day and night conditions, helping us evaluate photolytic and non-photolytic effects on attenuation. Water samples collected using a Lagrangian sampling scheme were analyzed through non-targeted suspect screening with a high-resolution UHPLC-Orbitrap-MS/MS instrument. The sensitivity ranking for over 100 micropollutants, based on their real-world susceptibility to photolysis, established in this study provides a foundation for future work aimed at more precisely quantifying biodegradation processes. The results improve our ability to characterize micropollutant attenuation in natural aquatic systems and will enhance the accuracy of estimates regarding their environmental persistence and fate.

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3.08.P-Th126 Different Factors Affecting the Seawater Sample Processing for the Trace Analysis of Organophosphate Triesters and their Metabolites: Application to the Loire Estuary (France)

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Organophosphate (tri)esters (OPEs) are high production volume chemicals currently in use as additives and/or flame retardants in a variety of plastics and polymers and are considered as contaminants of emerging concern widely distributed in the environment. Their environmental degradation can lead to the formation of the corresponding organophosphate diesters (diOPEs). Therefore, suitable analytical methods for both tri- and di-OPEs are needed for a comprehensive assessment of their environmental occurrence, especially in marine environments where knowledge on the presence and distribution of these pollutants is still scarce. Most studies rely on different sample treatment methods to detect and quantify tri- and di-OPEs in surface waters due to the differences in their physicochemical properties (e.g., Kow values, anionic nature of diOPEs). In this study, we investigated 3 factors affecting the recovery of 19 triOPEs and 6 diOPEs from seawater samples in a single step method based on SPE. We used a 33 factorial experimental design to evaluate the influences of the stationary phase, elution solvents and the washing and drying steps on the SPE performance. Target compounds were analyzed by isotopic dilution LC-MS/MS. Differences in the recovery rates were related to the physicochemical characteristics of the compounds; better recoveries were found for diOPEs using mixed mode WAX cartridges while DSC-NH₂ showed better performances for linear alkyl triOPEs. The best extraction protocol consisted on HLB SPE followed by an acid wash and drying step and elution with MeOH and ethyl acetate. This method was then validated by means on native OPE spiking experiments and later successfully applied for the simultaneous determination of tri- and diOPEs in 12 seawater samples from the Loire estuary (north west France). The sample sites included highly industrialized areas and near and offshore samples from touristic areas. Results showed up to 20-fold higher levels for certain OPEs (e.g., TEP, TBOEP) in the industrialized area with respect to reference offshore samples, and a widespread presence of aryl OPE and DPhP in seawaters. The developed method may improve the performance of monitoring programs for OPEs in marine environments allowing the inclusion of diOPE without significant increases in economical and temporal costs of the analytical process.

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3.08.P-Th127 Exploring the Effects of PFAS on Wild Populations of Mosquitofish: Focus on Behaviour and Reproduction

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The widespread contamination of surface and groundwater with per-/poly-fluoroalkylated substances (PFAS) is alarming due to their persistence, bioaccumulation, and adverse effects on living organisms. This work takes advantage of the naturally occurring high concentrations of PFAS detected in some areas of the Veneto region (Italy) caused by extensive environmental contamination. The aim is to assess the impact of PFAS in the wild using the mosquitofish (*Gambusia holbrooki*), a small freshwater fish, as a model organism. Mosquitofish is an invasive species that inhabits small rivers and streams worldwide. We have investigated the effects of PFAS exposure in polluted vs non-polluted areas on phenotypic traits, including life history, reproduction, and behaviour, as well as bioaccumulation in wild populations (complementary -omics analyses are planned to explore gene expression changes). Field sampling has been conducted across 8 sites, spanning regions with high and low PFAS contamination, to compare the ecological and physiological responses of resident mosquitofish populations. Chemical analyses of water samples were performed to quantify PFAS concentrations in the water at the moment of sampling (long-term data are available from ongoing assessment by local authorities), as well as bioaccumulation in males and females. Behavioural, reproductive, and life-history traits were assessed. Behavioural analyses included sexual behaviour, risk-taking behaviour and exploration (using an open-field test) repeated to identify population-level effects, but also within- and among-individual differences. Reproductive and life history traits included sperm number and quality, body size, and reproductive allocation. By investigating the chronic and multigenerational effects of PFAS (mosquitofish populations have likely been exposed for at least 30 generations) in natural conditions, the project captures the complexity of real-world scenarios, including interactions with other stressors such as additional contaminants, fluctuating environmental factors, and ecosystem dynamics. The comprehensive dataset generated will enhance our understanding of

how wildlife responds to PFAS contamination at multiple biological levels, paving the way for future research in alternative assessments of PFAS (and possibly other ED pollutants) and conservation strategies.

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3.08.P-Th128 Non-Target Analysis of Wastewater Treatment Plant Samples: Comparison of Fingerprint of Inflow and Outflow Water

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Wastewater treatment plants (WWTPs) are fundamental for pollutants removal from wastewater. However, the WWTP removal efficiency is very low for many xenobiotic compounds and, based on the European legislative framework (i.e., Urban Wastewater Treatment Directive), few compounds of those produced and used are routinely monitored in WWTPs inflow and outflow samples. This lack of suitable removal technologies and of knowledge of the full spectrum of contaminants potentially present in wastewater lead to an increase of the uncertainty in evaluating the risk for the aquatic ecosystem.

While traditional target approaches are feasible for assessing a limited number of compounds, they become very expensive and challenging due to the numerous compounds typically present in wastewater samples. On the contrary, non-targeted analysis (NTA) is a promising approach that allow the characterization of the chemical composition of a sample without prior knowledge of its chemical content and resulting in dataset containing both known and unknown compounds.

Here, we conducted a study on inflow and outflow samples from a WWTPs located in Como (Northern Italy) characterized by a population equivalent of 168000, and with various inlet sources. Composite 24-h samples of influent and effluent wastewater were collected for 1 day, following the water path in order to collect the same entering water which went through the 14-h treatment. Samples were extracted by Solid Phase Extraction (SPE) and analysed with an ultra-high performance liquid chromatography (UHPLC) coupled to a high-resolution mass spectrometer (HRMS) (Orbitrap Exploris 120). The raw data were then analyzed with Compound Discoverer software (Thermo Fisher) for untargeted analyses using a dedicated workflow. Data were manually validated applying some filters in order to obtain a confidence identification level 2a - probable structure. Many compounds were identified in inflow samples, while compounds in outflow samples were much lower. More than 70% of the identified compounds were industrial chemicals. Other categories were represented by pesticides, personal care products, and pharmaceuticals.

This study highlighted the importance of this approach in discovering xenobiotic diversity in wastewater influent and effluent, identifying also compounds not included in the current monitoring lists, but that can pose a risk to the aquatic ecosystem.

3.08.P-Th129 Machine Learning-Driven Forecasting of Organic Micropollutants in Aquatic Environments

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The contamination of aquatic ecosystems by organic micropollutants (OMPs), including pharmaceuticals, personal care products, hormones, pesticides, and washing agents, has raised significant environmental and public health concerns due to their bioaccumulative and toxic properties. The increasing levels of OMPs in freshwater bodies have prompted the enactment of chemical regulations to assess and mitigate associated risks. However, the detection and quantification of OMPs often require advanced and costly analytical techniques, posing challenges for comprehensive monitoring efforts.

This project leverages machine learning (ML) as a transformative tool to address these challenges by developing predictive models trained on datasets containing known OMP concentrations and corresponding environmental parameters. These environmental parameters, such as precipitation, water temperature, and flow, are obtained from trusted sources and matched to legacy grab samples. Since obtaining these auxiliary environmental parameters does not require additional fieldwork, these models can facilitate cost-effective prediction of OMP levels.

Additionally, the models highlight critical environmental variables to enhance future sampling and data collection campaigns. Our approach employs techniques from the field of explainable ML, such as Grad-CAM (Gradient-weighted Class Activation Mapping) and SHAP (SHapley Additive exPlanations), to

identify which auxiliary environmental parameters are most crucial for prediction.

These models will inform decisions on necessary treatments at wastewater treatment plants (WWTPs) and upstream interventions, ultimately supporting the national goal of achieving a non-toxic environment. This work has significant implications for enhancing the protection and sustainability of aquatic ecosystems in Sweden and beyond, ensuring the health of both the environment and human populations.

3.08.P-Th130 Accelerating Effect-Directed Analysis Through Constructing Non-Target HRMS Libraries for AhR Agonists by Deep Learning

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Sediments are influenced by chemical mixtures. Effect-directed analysis (EDA) is promising for identifying bioactive contaminants, especially when combined with non-target screening (NTS), but time-consuming workflows have hindered its large-scale applications. Herein, we developed an event-driven taxonomy (EDT)-Screening strategy to effectively identify suspect and non-target bioactive contaminants in sediment, taking aryl hydrocarbon receptor (AhR) activity as an example. To accelerate EDA and NTS workflows, the EDT-Screening strategy integrated fractionation, bioassay, and identification into one step by embedding two newly constructed effect-based spectral libraries to LC-HRMS screening templates. The event driver (ED) library was assembled from data-mined AhR-active compounds, and event driver ion (EDION) library contained effect-related fragment ions generated by deep learning models. The AhR-ED library identified more AhR-agonists with lower incidence of false positives for tentative candidates compared to the ChemSpider database. Furthermore, AhR-EDION facilitated the identification of additional AhR agonists in sediment, especially industrial intermediates and transformation products that were often overlooked due to lack of priori knowledge. This highlighted the effectiveness of the EDT-Screening strategy in expanding the pool of unknown unknowns in bioactive contaminants. In conclusion, the construction of effect-based HRMS libraries provided a rapid solution for the identification of bioactive contaminants in complex chemical mixtures.

3.08.P-Th131 Proving That Aquatic Toxicity Testing Is Technically Not Feasible: Not So Simple. Case Study of UVCB Emulsifiers

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According to OECD 23, difficult to test substances are defined, but not only, as being either poorly or sparingly water-soluble, volatile, hydrophobic, multi-component, and/or highly adsorptive. For most of these substances, technical adaptations are available to allow an accurate measurement of the substance in water to perform a robust study.

In a regulatory context, Annex XI section 2 of REACH stipulates that testing for a specific endpoint may be omitted if it is technically not possible to conduct the study because of the properties of the substance. REACH R.5, section 5.2.2, however, states that, for poorly water-soluble substances, different test durations and alternative test methods need to be considered, i.e., chronic testing instead of acute testing. In some instances, all reasonable technical adaptations may have been exhausted and the substance is still not measurable in water. The key question is, to what extent do analytical method and sample preparation optimization need to be performed to consider that, for a particular test substance, a study is technically not feasible?

Here we explore this question with four substances under OECD 201. Substances were two glycerides (mixtures of glycerol mono ester, acetylated, diacetylated) and two polyols (mixtures of Di-, Tri-, glycerol), with chain length variation between C12 and C24 for all substances. Water solubilities were between 45 µg/L and 91 µg/L in pure water. MS/MS methods were used leading to Limit of Detection < 5 µg/L. Water Accommodated Fraction parameters were optimized to maximize water solubility whilst avoid microdroplets and all prepared under sterile conditions to avoid biodegradation.

In one trial out of six, a substance was detected below the water solubility. Once the trial was repeated with a longer settling period (24h instead of 4 h), no substance was detected. This led to the hypothesis that the substance was so insoluble, that once there was enough time of settlement in water, the substance reaggregated to form microdroplets instead of staying solubilized. The same trials were performed with the other substances. Similarly, no substance was detected leading to the conclusion that aquatic testing with these substances was not feasible.

3.08.P-Th132 From Microalgae to Gastropods: Understanding the Kinetics and Toxicity of Silver Nanoparticles in Freshwater Aquatic Environment

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Silver nanoparticles (AgNPs) are increasingly used in various consumer products and industrial applications, raising concerns about their potential environmental impact on aquatic ecosystems. This study aimed to evaluate the effects of AgNPs on both a primary producer, diatom *Cyclotella meneghiniana*, and freshwater gastropod *Lymnaea stagnalis*, by assessing waterborne, foodborne, and combined exposure routes. Our objective was to determine whether AgNPs disrupt the physiology of diatoms and whether these disruptions and Ag accumulation transfer to higher trophic levels, causing physiological stress in gastropods. We found that AgNPs exposure in *C. meneghiniana* significantly disrupted trace metal homeostasis, notably reducing iron, copper, and manganese concentrations while increasing zinc levels. Additionally, diatoms secreted extracellular polymeric substances, which likely served as a protective mechanism against NPs toxicity. However, the disruption of trace metal balance raised concerns about the potential transfer of Ag and physiological disruptions to organisms that feed on these primary producers. The alteration in trace metal concentrations, particularly the depletion of critical cofactors involved in photosynthesis and antioxidative defenses, suggests that diatom metabolic function might be compromised, potentially affecting nutrient cycling and energy transfer within aquatic ecosystems. In *L. stagnalis*, waterborne exposure resulted in the highest silver accumulation, followed by rapid depuration. However, this clearance process triggered oxidative stress during depuration, as evidenced by increased malondialdehyde levels. Foodborne exposure, simulating trophic transfer from algae to gastropods, resulted in slower silver depuration, prolonged retention, and increased hemocyte mortality, indicating significant immune stress. Combined exposure led to the most severe oxidative stress during uptake, with increased reactive oxygen species production and protein synthesis in hemolymph, suggesting an intensified physiological response. These findings demonstrate that AgNPs not only disrupt primary producers but could also transfer across trophic levels, leading to significant physiological stress and toxicity in higher organisms. This study emphasized the importance of considering multiple exposure routes in environmental risk assessments and highlights the potential for AgNPs to bioaccumulate and possibly biomagnify within aquatic ecosystems.

3.08.P-Th133 Prediction of the Surface Charge of TiO₂-Nanoparticles in Surface Waters

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Anthropogenic nanoparticles, both incidental and engineered, are released into the environment in increasing amount, with unclear ecological consequences for aquatic ecosystems. Understanding how nanoparticles interact with natural surfaces is complicated by limited knowledge of their surface chemistry in real-world conditions. This study focuses on the ζ -potential (surface charge) of nanoparticles in natural waters and propose a model to predict how it is influenced by environmental factors. Five types of TiO₂-nanoparticles, commonly used in commercial products, were exposed to 20 surface water sites across Germany and Switzerland, over three seasons. The ζ -potential was measured using laser Doppler velocimetry, and we trained random forest models to predict ζ -potential from water composition. The importance of the environmental factors for the prediction was assessed using permutation tests and individual conditional expectation (ICE) plots. Further, surface characterization was done using infrared spectroscopy (IR), time of flight secondary ion mass spectrometry (ToF-SIMS), and X-ray photoelectron spectroscopy (XPS).

The concentration in divalent cations (Ca²⁺ and Mg²⁺) was the most important factor influencing the ζ -potential, with higher ion concentrations reducing surface charge. Once a threshold was reached, further increases in ion concentration had no effect. Surprisingly, pH had little impact, possibly due to a narrow pH range in natural waters and the shielding effect of organic matter on surface charge. DOM composition influenced ζ -potential, confirmed by the importance of fluorescence data for the models, showing that organic coatings modulate nanoparticle behavior. XPS and ToF-SIMS analysis confirmed the presence of organic coatings dependent on water composition.

Our results demonstrate the potential of our approach to investigate the surface properties of nanoparticles in natural waters while accounting for the complexity of the environmental medium. The concept could be applied to other nanoparticles, including natural ones, and to investigate other physico-chemical properties of the coatings such as composition, thickness or stability. It could also be applied to produce environmentally coated nanoparticles which could serve as more realistic test items in ecotoxicological studies, for instance.

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3.08.P-Th134 Variation in Horizontal Distribution of Microplastic Polymers Exceeding 20 Microns in Tokyo Bay Sediments: Effects of Density and Particle Size

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Understanding the behavior of microplastics (MPs) in aquatic environments is essential for reducing their contamination levels in the future. Large quantities of MPs can enter bays near densely populated urban areas and ultimately settle into sediments. However, there are currently no comprehensive reports on the horizontal distribution of smaller MP polymers in bay sediments over a wide range.

In this study, we investigated the concentrations and size distributions of MPs greater than 20 microns in sediment samples from 10 sites in the northern region of Tokyo Bay, one of the most heavily contaminated bays in Japan. Sediment matrices were removed using a hydroperoxide-based Fenton reaction and density separation with sodium iodide. Extracted samples containing MPs were filtered onto a silicon membrane, and all MPs across the entire membrane area were automatically analyzed using micro-FTIR imaging techniques and our custom identification program, YCALOS, for accurate identification and quantification. MP concentrations in sediments ranged from 38.2 to 242.5 pieces per gram (dry weight), with 14 different polymers identified, including ABS, alkyd resin, AS resin, ethylene vinyl acetate (EVA), polyamide, polyethylene (PE), PET, PMMA, polypropylene (PP), polystyrene, polyurethane, polyvinyl acetate, and polyvinyl chloride.

Using the Kriging interpolation method, we generated contour diagrams for each polymer, revealing distinct sedimentation patterns that varied by MP size distribution and density. Polyethylene terephthalate particles and fibers, as high-density MPs, tended to settle close to river mouths, forming smaller hotspot areas. In contrast, low-density MPs like PE and PP were widely dispersed in the bay's central area, with hotspots for particles smaller than 100 microns. These findings suggest that high-density MPs (greater than 1.0 g/cm³) settle rapidly, while low-density, smaller-sized MPs disperse widely within the bay, settling only when their density increases above 1.0 g/cm³ through aggregation with biofilms, algae, or other mineral particles.

These insights enhance our understanding of the mechanisms driving MP sedimentation in coastal environments, providing crucial information for managing and mitigating MP pollution in aquatic systems.

3.08.P-Th135 Distribution Characteristics and Risk Assessment of Microplastics in Surface Water and Groundwater from Hetao Irrigation District

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Hetao Irrigation District is a major grain and oil seed production region and one of the top three largest irrigation districts in China, which is facing complex challenges for green development of agriculture. The large-scale use of agricultural mulch film in the irrigation district have been more than 30 years, while pollution in the surface water and groundwater caused by microplastics from agricultural mulch film was lack of systematic research. In order to investigate the distribution characteristics and risk of microplastics in surface water and groundwater from the irrigation district. Wuyuan County was selected as the objective area, which was one of the first pilot areas of agricultural non-point source pollution control and supervision guidance in China, surface water and groundwater samples were collected. Thermoextraction and desorption-gas chromatography/mass spectrometry were used to determine five types of microplastic polymers including Polyvinyl chloride, Polyethylene terephthalate, Polyethylene, Polystyrene, and Polythene in these water samples. The results showed that the concentrations of microplastics in surface water samples from each drainage ranged from ND to 317.1±2.1 µg L⁻¹, Polyvinyl chloride was the most predominant polymer in these surface water samples, accounting for 44.19%. The concentrations of microplastics in the groundwater samples ranged in ND ~ 44.8 ± 7.9 µg L⁻¹; Polythene was the most predominant polymer in the groundwater samples. Polymer hazard index was used to evaluate polymer-specific risks, Polyvinyl chloride was found to be the high risk polymer. These results will provide a cutting-edge research basis for preventing and controlling microplastics pollution in the irrigation district.

3.08.P-Th136 Adsorption of Benzophenone-3 and Octocrylene UV Filters on Polyethylene: Analysis by HPLC-MS/MS and Voltammetry with Screen-Printed Electrodes

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Microplastics (MPs) are pollutants of global concern, posing a persistent threat due to their accumulation and low degradation rates in the environment. Acting as transport vehicles, MPs adsorb various contaminants, facilitating their accumulation in aquatic ecosystems. This issue is exacerbated by emerging contaminants such as UV filters (UVFs), particularly in coastal areas, where combined exposure poses significant environmental risks. This study focuses on determining the adsorption capacity of polyethylene

(PE) for benzophenone-3 (BP3) and octocrylene (OC), evaluating the influence of parameters such as pH, temperature, and exposure time. A central composite design (CCD) was employed to optimize resource efficiency during experimentation. Controlled experiments were conducted in both pure water and synthetic seawater. High-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) was applied to accurately quantify UVF concentrations, providing high sensitivity and specificity in chemical detection. In parallel, differential pulse adsorption stripping voltammetry (DPAdSV) using screen-printed electrodes (SPE) was applied for electrochemical analysis, offering a rapid and cost-effective approach to monitor adsorption dynamics.

The findings revealed that OC exhibited a stronger affinity for PE than BP3, attributed to its higher octanol-water partition coefficient ($\log K_{ow}$ 6.88 vs. 3.79), indicating greater compatibility with non-polar environments. Among the variables studied, exposure time emerged as the most significant factor influencing adsorption in aqueous solutions. In contrast, pH and temperature had minimal impact in pure water but were significant in synthetic seawater, particularly for BP3, due to electrostatic and solubility effects (data for OC in synthetic seawater is currently being processed). The dominant interactions driving adsorption were hydrophobic in nature. Notably, the results from electrochemical analyses using differential pulse adsorption stripping voltammetry (DPAdSV) closely aligned with those obtained via HPLC-MS/MS, establishing SPE as a reliable and efficient method for in situ BP3 detection. This study highlights the utility of CCD in identifying critical variables and optimizing experimental designs to better understand the adsorption behavior of UVFs on MPs.

3.08.P-Th137 Indicators and Impacts of Microplastics Released from Traffic Paints and Paved Roads into Surface Waters, Detected Using an Automated Microplastic Preparation System

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Despite the widespread use of traffic paints and polymer-modified asphalt containing various microplastics (MPs) in road markings and pavement worldwide, accurately detecting and quantifying their release into surface waters including rivers, lakes, and seas remains a considerable challenge. This study employed both an Automated Microplastic Preparation device (AMP) and a General Manual Preparation method (GMP) to measure the concentrations and size distributions of 20 polymer types of MPs larger than 300 μm in 17 surface water samples from rivers and a lake affected by road dust, as well as 6 samples from Tokyo Bay. Observed MP concentrations across all sampling sites ranged from 0.06 to 25.8 pieces/ m^3 . Notably, polyethylene-ethyl acrylate (PEA) copolymer and urethane alkyd (UA), commonly used as additives in asphalt and traffic paints, were detected only via AMP, particularly in river waters, while GMP failed to detect these polymers. Their maximum concentrations reached 4.2 pieces/ m^3 . Combined, PEA and UA accounted for between 5.9% and 45.2% of total MPs in river water contaminated by road dust under dry weather conditions.

Principal component analysis of polymeric composition data revealed three primary groups: the first consisting of additive polymers like PEA and ethylene vinyl acetate, the second of traffic paint polymers such as UA and alkyd resin, and the third of common sea water MPs like polyethylene and polypropylene. Additionally, annual loads of PEA and UA into Tokyo Bay showed a significant positive correlation with road areas in the catchment areas of sampling sites. These findings indicate that PEA and UA may serve as reliable indicators of MP contamination arising from road markings and pavement.

3.08.P-Th138 Microplastics in Elbe River Sediments Analyzed by Thermal Extraction-Desorption Gas Chromatography-Mass Spectrometry (TED-GCMS)

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Microplastic pollution is a globally emerging environmental concern, with sediments serving as major sinks for certain polymers in aquatic systems. Traditional environmental analysis of microplastics typically involves spectroscopic methods, such as Fourier Transform Infrared and Raman spectroscopy. While effective for identifying microplastic morphology, these methods are time-consuming and may overlook quantitative (mass) data. In contrast, methods like Thermal Extraction-Desorption Gas Chromatography-Mass Spectrometry (TED-GCMS) can provide valuable mass-based measurements of microplastics. In this study, we applied this thermal method to investigate microplastic pollution in

sediments from the Elbe River. Twenty-seven sediment samples were collected along the river, covering the Czech Elbe, the free-flowing inland Elbe, the tidal Elbe, and the North Sea. After density separation and digestion, sediment samples were analyzed. Microplastic patterns covering seven common polymers, i.e., polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyamide (PA), polylactic acid (PLA), and polymethyl methacrylate (PMMA), will be presented.

3.08.P-Th139 Development of an Analytical Method for Measuring Fine Rubber Particles Exceeding 20 Microns in Sediment Using Micro-FTIR Imaging Techniques

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Synthetic rubbers present in atmospheric, aquatic, and terrestrial environments are of increasing concern due to their ecological impacts on wildlife and potential health risks to humans. Tire wear particles, in particular, have been widely studied and are typically analyzed using ICP-MS, Py-GCMS, and vibrational spectroscopy. Although vibrational spectroscopy provides non-destructive and rapid analysis, measuring fine rubber particles smaller than 1 mm remains challenging due to weak spectral signals and limited effective pretreatment methods for environmental samples, making it difficult to isolate and analyze such particles without physical handling limitations.

In this study, we developed a simultaneous analytical method to detect nine types of elastomer particles larger than 20 microns in sediment using micro-FTIR imaging. The elastomers analyzed included Styrene-Butadiene Rubber, Tetrafluoroethylene-Propylene Elastomer, Ethylene-Propylene Rubber, Chloroprene Rubber, Chloro-Sulfonated Polyethylene Rubber, Isobutylene-Isoprene Rubber, Silicone Rubber, Acrylonitrile Butadiene Rubber, and Ethylene-Propylene-Diene Terpolymer Rubber all common on road surfaces in Japan. A novel pretreatment procedure was established to digest sediment samples from Tokyo Bay without hydroperoxide, as hydroperoxide could dissolve the elastomers. Following digestion, density separation was performed similarly to the method for microplastics reported by Kameda et al. The separated samples were then filtered onto a silicone membrane, and the spectral data for all particles across the membrane were automatically collected using micro-FTIR imaging.

For identification, we employed a newly developed software, YCALOS, which accurately identified the nine elastomers, overcoming the limitations of existing software in handling small particles due to interference from sample matrix peaks and noise. This advanced analytical method for detecting elastomers in sediment could also be applied to surface water, wastewater, and atmospheric samples, broadening its potential environmental applications.

3.08.P-Th140 Automatic Sample Preparation Device for Microplastics Analysis

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Microplastics have been found in every environmental compartment in urban and remote areas wherever they have been studied. Even though the omnipresence of microplastics in the environment is acknowledged, different approaches in sampling, sample preparation, and particle identification/quantification hamper comparison between studies. Since the environmental samples have a complex particulate matrix, the several laborious treatments must be performed to remove interfering particles prior to chemical analysis.

To lighten the workload and improve the process repeatability, Shimadzu Corporation has developed an automatic sample preparation device (MAP-100) for the isolation of microplastics from environmental samples. The MAP-100 automatically performs a multistep preparation including chemical oxidation, serial (1 to 3) density separation, and final filtration. The efficiency of sample preparation can be enhanced with an integrated magnetic stirrer and temperature control. The lower size limit of MAP-100 is 300 µm. In this study, the performance of the novel sample preparation device was explored.

For a performance test, three types of surface water samples were collected with a manta trawl (mesh size 300 µm) from Lake Saimaa, the Gulf of Finland and the River Vantaa. Hydrogen peroxide (30%) was used for the oxidation of organic material in samples, whereas mineral particles were separated in a saturated sodium iodide solution (density 1.8 g/cm³). The collected samples were spiked with the reference plastics that represented different sizes and densities: 355-425 µm polyethylene and poly(methyl methacrylate), 3 mm polyoxymethylene and 4 mm polypropylene. Laboratory blanks were analyzed along with the field samples. The unknown particles were analyzed with a Fourier Transform Infrared spectrometer equipped with an attenuated Total Reflectance measurement unit.

The performance of the MAP-100 was evaluated in terms of recovery rate, sample contamination, and the removal efficiency of sample matrix. The recovery rates were derived for microplastics close to the lower cutoff size of the MAP-100 and to the upper size limit of microplastics. If the performance is satisfactory, the MAP-100 is a very appealing tool for monitoring microplastics in surface water.

3.08.P-Th141 Development of a Portable and Cost-Effective Sampling Device for Microplastics in Water, Utilizing Household Water Filtration Systems

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Sampling microplastics (MPs) in surface waters such as rivers, lakes, bays, oceans, and even tap water typically requires large and heavy equipment, including neuston nets, plankton nets, and large pumps. This is due to the substantial water volumes needed for analysis: for instance, European guidelines recommend sampling 1000 L of tap water, while hundreds of liters are often necessary to collect MPs larger than 20 μm in environmental waters. These methods are labor-intensive, time-consuming, and challenging to deploy widely. Specialized filtration systems employing pumps and disc membranes have been developed, but these systems are often expensive, custom-made, and inaccessible for broad application.

Furthermore, detecting MPs smaller than 10 μm , critical for assessing ecological and human health risks, presents additional challenges. Conventional methods, such as neuston and plankton nets, are unsuitable for capturing MPs in this size range.

To address these issues, this study aimed to develop a portable, cost-effective sampling device for MPs in water samples. We utilized a commercially available household water filtration system (Mitsubishi Chemical Cleansui Corporation), which is easily accessible via online platforms such as Amazon and Yahoo. The system employs hollow fiber membranes capable of concentrating MPs larger than 0.2 μm into a collection vessel. Its compact size and lightweight design make it suitable for use in diverse environments, including research vessels, riversides, kitchens, and bathrooms.

Our investigations demonstrated that the system could filter 300 L of river water, seawater, or tap water in just 30 minutes without requiring membrane replacement during the filtration process. Contamination from the filtration system itself was minimal, with MP concentrations from the household membranes significantly lower than the concentrations of MPs collected in the samples. Additionally, we developed an efficient extraction method to recover MPs from the membranes while minimizing contamination during the procedure.

3.08.P-Th142 Small Microplastics (<100 μm) in Wet Depositions: First Micro-FTIR-NIR Analysis and Creation of NIR Libraries

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SMPs (small microplastics <100 μm) have become a significant environmental concern due to their widespread presence and, due to their smallest size, they are posing a significant threat to the biota and human health. However, SMPs are still often overlooked, and, due to different pretreatment and analytical methods, the results of the few studies on SMPs can be challenging to compare, leading to discrepancies in data quality and reliability. Besides, it is crucial to cross-validate different analytical methods for assessing SMPs, to ensure consistent and reliable results, and enable more robust evaluations of environmental impact and potential negative implications for human health. In scientific literature, a very low number of MP studies employed the NIR spectroscopy technique and there is more lack of knowledge in the use of this technique for the smallest size (SMPs) analysis and the removal procedures for the interferences assessment (organic, inorganic and biological compounds) from real environmental samples. In our study, SMPs in different size ranges were analyzed employing two analytical supports (e.g., BaF₂ support and ANODISC filter, respectively) with Micro-FT-NIR spectroscopy to create new libraries. Then, a comparison with a real environmental sample (e.g., a wet deposition from the urban area of Mestre, Venice, Italy) filtered after an oleoextraction and purification procedure previously developed (Corami et al., 2021) on ANODISC filter was analyzed using the same libraries. Different SMP typologies were confirmed using FT-NIR spectroscopy in wet depositions with high matches (<90%). Hence, ANODISC filters were confirmed to be a suitable support for SMP analysis in FT-NIR spectroscopy, opening possibilities for expanding the cross-validation techniques approach for SMP detection providing reproducible and reliable data in real environmental samples. Further, FTI-NIR spectroscopy is confirmed to be fast for real-time SMP analysis, allowing the polymer characterization of a wide range of polymers with an appropriate pretreatment methodology for real environmental samples.

3.09.A Future of Suspect and Non-Target Screening to Monitor Emerging Contaminants in the Environment

3.09.A.T-01 Integrating TIMS-PASEF with LC-HRMS and Biotransformation-Driven Suspect and Non-Target Screening Workflows to Address Key Challenges in Xenometabolome Analysis

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The impact of xenobiotics in the aquatic ecosystem is evaluated in more depth when the whole xenometabolome (xenobiotics and their biotransformation products (bio-TPs)) of aquatic organisms is studied. Despite the technological advancements in LC-HRMS, the identification of bio-TPs remains challenging. This study addresses the analytical (e.g., isomeric bio-TPs, low-intensity bio-TPs, and absence of MS/MS data) and data processing (complex unknown bio-TPs) challenges of comprehensive xenometabolome assessment. Using ZFE exposed to benzotriazoles (BTs) and triclosan as complex case studies we aim to answer the following research questions: (1) How can we generate extensive, multi-dimensional analytical evidence to support challenging identification cases, and (2) How can we best leverage the produced analytical evidence, along with the inherent biotransformation context (known structure of the parent compound and biotransformation reactions), to prioritize and annotate unknown bio-TPs with high confidence.

To achieve this, we combined complementary analytical dimensions (RPLC, HILIC, TIMS) to extend the available analytical space. The potential of Trapped Ion Mobility Spectrometry (TIMS) combined with Parallel Accumulation Serial Fragmentation (PASEF) and HRMS to overcome limitations regarding isomer separation, spectral complexity, sensitivity, and MS/MS coverage was evaluated. Finally, a biotransformation-oriented data processing workflow was established, integrating suspect screening, non-target screening and the inherent characteristics of biotransformation experiments to identify bio-TPs with high confidence.

Our results demonstrate that TIMS effectively separated isomeric bio-TPs (OH-sulfate-4MeBT) and enhanced S/N ratios for improved detection of low intensity features. PASEF delivered comprehensive MS/MS coverage (>70% of precursors) without compromising sensitivity, enabling confident identification even for low-intensity bio-TPs (OH-glucuronide-4MeBT). The developed data processing workflow, combining suspect and non-target screening within silico tools, facilitated the annotation of complex bio-TPs, including those formed through multiple sequential biotransformation reactions. Overall, this approach significantly enhances our ability to characterize the xenometabolome with confidence, and ultimately improve xenobiotic risk assessment.

3.09.A.T-02 Identification of Novel Highly Polar Sulfonated Disinfection By-Products in Drinking Water using Supercritical Fluid Chromatography-High Resolution Mass Spectrometry

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The generic methods to characterize disinfection by-products (DBPs), gas chromatography or reversed-phase liquid chromatography coupled to high resolution mass spectrometry (HRMS), can result in significant analytical gaps by overlooking the very polar fractions of DBPs. For the first time in this study, we applied an alternative method, supercritical fluid chromatography-HRMS, to revisit DBPs in drinking water treatment plants and distribution networks. In total 15 novel highly polar DBPs were identified as the sulfonated derivatives of haloacetonitriles, haloacetamides, and haloacetaldehydes. Regardless of the lack of analytical standards, we were able to conduct the structural confirmation and quantification of these newly identified DBPs by preparing their mixture via chlorination of a precursor compound and by combining nuclear magnetic resonance spectroscopy analysis with SFC-HRMS analysis. The concentrations of total haloacetonitrilesulfonic acids and haloacetaldehydesulfonic acids in drinking water were estimated to be up to 50 and 800 ng/L, respectively. Considering the stronger toxicity of haloacetonitriles, haloacetamides, and haloacetaldehydes than that of the regulated DBPs, these newly found sulfonic acid derivatives may also pose a health risk.

3.09.A.T-03 Extensive Chromatographic Platform Comparison (LC-, HILIC-, IC-, SFC-HRMS) for Polar Contaminants in Groundwater

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The analysis of emerging contaminants and their transformation products (TPs) in the aqueous environment is a major challenge due to the wide chemical space that needs to be captured analytically. While most studies focus on established liquid chromatography coupled with high-resolution mass

spectrometry (LC-HRMS) for non-target and suspect screening, there is an increasing need for the addition of complementary chromatography. In particular, the inclusion of very polar compounds due to their high mobility and threat to drinking water resources is needed.

Our study aims for a comparison of a wide range of separation methods (LC-, HILIC-, IC-, and SFC-HRMS) for improved chemical space coverage in groundwater. Aliquots from a standard mixture (>100 compounds) and a groundwater sample from Denmark enriched by multiphase SPE were distributed between 4 laboratories and measured with 10 different chromatographic methods all coupled to HRMS. Data was acquired both in positive and negative polarity with data-dependent (DDA) and data-independent (DIA) acquisition to maximize fragmentation information.

After feature detection and several filtering steps, the detected features were aligned across platforms by accurate mass and MS/MS spectral similarity to be able to compare detections across platforms and investigate similarities and complementarities. Furthermore, identified compounds from every individual method were compared to investigate the amenability of certain compound classes to certain platforms (e.g., which compounds are unique to SFC-HRMS). While LC-HRMS was able to cover only half of the standards, the addition of the other platforms increased the compound coverage by an additional 40%. SFC-HRMS was able to cover the most compounds due to its ability to retain both very polar compounds but also more hydrophobic ones. IC-HRMS could also detect several unique compounds (e.g., Ethephon) not covered by other platforms. Our approach highlights both advantages and challenges arising when combining HRMS data from many complementary chromatographic methods.

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3.09.A.T-04 Chemical Profiling and Toxicity of the Airborne Exposome in Indoor and Outdoor Environments using Polydimethylsiloxane (PDMS) Foam Passive Samplers

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The chemical exposome encompasses all environmental chemical exposures an individual encounters from conception to death. The air we breathe is a key pathway of exposure, and contributes to chronic disease and premature mortality around the world, yet its chemical composition is largely unexplored in most human environments. To advance airborne exposomics and broaden the chemical space examined, we developed and tested polydimethylsiloxane (PDMS) foam for passive air sampling in indoor and outdoor environments. This material captures both (semi-)volatile organic compounds and particulate matter and can be compatible with toxicity assays, facilitating molecular discovery, quantification, and toxicity assessment for a broad spectrum of airborne chemicals and their mixtures. We specifically optimized and validated GC-HRMS and LC-HRMS sample preparation methodologies for using over 200 prioritized substances from 20 different chemical classes, and demonstrate a method to assess the cytotoxicity of extracts in primary human lung fibroblasts. Together, these methods allow for comprehensive chemical analysis and toxicity assessment for the same passive air samples. Application to an indoor pilot study, with samples collected over eight-weeks, allowed to detection of 21 LC and 46 GC target analytes with concentrations in sampler ranging between 0.003 297.7 ng/cm², including the presence of 23 PCBs. LC- and GC- amenable chemicals such as DEET, tris(2-butoxyethyl) phosphate, or tributyl phosphate, were detected in both modes at similar concentrations, adding a confidence layer to the reported results. Additionally, suspect screening using MS-Dial software and public libraries (e.g., Mass Bank North America, or GNPS) allowed for the tentative identification of 39 (LC) and 62 (GC) chemicals at level 2. Finally, nontarget approaches such as feature-based molecular networking and network annotation propagation are being applied to further identify features of interest that can be linked with high cytotoxicity in human lung cells. PDMS foam disks have been deployed in over 200 homes across eight European countries as part of the Horizon Europe INQUIRE project. We will present novel results and preliminary data from this extensive sampling effort, highlighting the samplers' effectiveness in airborne exposure assessment, and toxicity evaluation to supports studies of the exposome and future chemical regulation.

3.09.A.T-05 Connecting Contamination Fingerprints and Toxicity Patterns in Source-Related Effluents using Non-Target Screening

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The SOURCES project of the European NORMAN network aims to explore the contamination patterns, toxicity profiles and identify effect drivers in different micropollutant sources discharging into the water cycle across Europe. Conventional chemical analysis offers only a partial view of these complex mixtures, making effect-based methods and non-target screening (NTS) essential to enable a more thorough chemical and effect evaluation. This study applies two prioritization strategies to link features detected in over 100 samples from sources-related effluents to their bioactivity in nine in vitro bioassays. The tested endpoints include: androgenic activity, estrogenic activity, neurotoxicity, thyroid hormone receptor activity, mutagenicity (two YG strains), oxidative stress, PXR activity and AhR activity. Following SPE extraction and analysis by HPLC-Exploris Orbitrap, the data was processed using MZmine 3 prior to feature prioritization. First, the MLinvitroTox toxicity prediction tool, based on MS/MS data, was used to filter relevant candidates for each bioassay endpoint. These candidates were then analyzed using PLS and Random Forest (RF) models to correlate chemical features with bioassay data, identifying the primary effect drivers. In total, 154,572 features were detected after intensity filtering and blank subtraction, with 997 and 251 features prioritized for androgenicity and oxidative stress, respectively. The RF models, with R^2 values of 0.67 and 0.30, helped identify the top 20 features linked to androgenic activity, primarily steroids and their derivatives, along with some anti-androgenic coumarins. For oxidative stress, O-glycosides were notably present in the top 20, though structural searches did not yield strong candidates. Preliminary results suggest that the observed effects are related to the prioritized compounds, particularly for androgenicity, while oxidative stress may be driven by the overall chemical mixture. The proposed approach enables significant prioritization and focuses identification efforts on a small group of candidates, thereby improving the efficiency of effect driver detection in monitoring studies. Further work will extend this approach to other endpoints and confirm candidates through spectral library searches and target screening.

3.09.B Future of Suspect and Non-Target Screening to Monitor Emerging Contaminants in the Environment

3.09.B.T-01 Non-Target Screening of Water Samples and Application of Different Prioritization Strategies

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Municipal wastewater treatment plants (WWTPs) are known sources of organic micropollutants in surface waters. Originally designed to treat biological waste, such as human urine and feces, WWTPs now face increasing challenges due to the widespread use of pharmaceuticals, personal care products, and household chemicals. These products introduce a diverse array of chemicals into wastewater. However, WWTPs are not yet fully optimized for the removal of these anthropogenic chemicals or their transformation products, resulting in their emission to the environment. In this study, we carried out an extensive sampling campaign collecting 60 consecutive 24h composite samples at one of the largest WWTPs in the Netherlands. We analysed these samples along with surface water samples using suspect- and non-target screening with reversed-phase liquid chromatography (RPLC) coupled to high-resolution mass spectrometry (HRMS). In order to be able to focus on the most relevant features and samples, we applied different prioritization strategies. A combination of multivariate statistics and a recently developed prioritisation model based on the prediction of structural alerts from tandem mass spectra (MS2) were used to prioritize features of interest for further investigation. The approach developed here using the combination of different prioritization strategies can be utilized in both environmental, exposomics, and human (bio)monitoring studies. They can rapidly highlight potentially significant features or samples that require further investigation, either by applying other in silico tools or through additional experimental work like targeted analysis. Additionally, findings from this study show that based on raw and unfiltered MS2 spectra, it is possible to predict whether detected features potentially contain specific structural alerts associated with toxic effects.

3.09.B.T-02 Prioritization of Unknown Features Based on Predicted Toxicity Categories

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Environmental samples are highly complex, containing a wide range of both known and unknown components. Liquid Chromatography coupled with High-Resolution Mass Spectrometry (LC-HRMS)

Non-Targeted Analysis (NTA) has become a critical method for studying these samples comprehensively. However, identifying the detected constituents poses a significant challenge due to the large number of features revealed by the analysis. To overcome this, prioritization strategies are often used to focus on the most relevant features for further investigation.

In this research, we developed an alternative prioritization approach that connects fragmentation and chromatographic data directly to aquatic toxicity categories, eliminating the need for individual compound identification. Recognizing that fragmentation data is not always available or well-characterized, we introduced two predictive models: a Random Forest Classification (RFC) model and a Kernel Density Estimation (KDE) model. The RFC model uses MS1 data, retention time, and cumulative neutral losses (CNLs) derived from fragmentation to classify fish toxicity categories when fragmentation data is available. In contrast, the KDE model relies solely on retention time and MS1 data for cases without fragmentation information.

Both models achieved accuracy levels comparable to structure-based prediction methods. We further validated their performance using an LC-HRMS pesticide mixture in a tea extract, where the CNL-based RFC model achieved an accuracy of 0.76 and the KDE model reached 0.61. These results highlight the models robustness and potential for real-world applications.

3.09.B.T-03 PubChemLite plus Collision Cross Section (CCS) Values for Enhanced Interpretation of Non-Target Environmental Data

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Finding relevant chemicals in the vast (known) chemical space is a major challenge for environmental and exposomics studies leveraging non-target high resolution mass spectrometry (NT-HRMS) methods. Chemical databases now contain hundreds of millions of chemicals, yet many are not relevant - and many relevant chemicals are missing. PubChemLite for Exposomics is an extensive collaborative, open science effort to provide a dynamic collection of chemicals for environmental, metabolomics and exposomics research, along with supporting information about their relevance to assist researchers in the interpretation of candidate hits. The PubChemLite for Exposomics collection is compiled from ten sections of PubChem, enhanced with patent, literature and annotation counts and predicted partitioning coefficient (logP) values, as well as predicted collision cross section (CCS) values using CCSbase to support ion mobility experiments. Predicted CCS are currently provided for 8 adducts ([M+H]⁺, [M+Na]⁺, [M+NH₄]⁺, [M+K]⁺, [M-H]⁻, [M+Na-2H]⁻, [M]⁺, [M]⁻). Monthly versions are archived on Zenodo under a CC-BY license, supporting reproducible research. A new interface has been developed for researchers to browse the collection, including several search functions and a per-chemical overview of chemical identifiers, the annotation content, chemical stripes on patent and literature data as well as tables of the predicted CCS values for the 8 adducts. In order to provide additional experimental CCS values to improve the coverage of CCS predictions for environmentally relevant chemicals, a collection of open experimental collision cross section values were compiled and added to PubChem, along with pipelines to add new data as received and update the corresponding compiled dataset. This contribution will describe the collaborative efforts to build the PubChemLite with CCS (PCL-CCS) versions, how this can support researchers in environmental/exposomics studies and explore known limitations and potential for future developments. The code (GitLab) and data behind these efforts are openly available (DOI: 10.5281/zenodo.4081056 and DOI: 10.5281/zenodo.6800138 redirect to the latest version of PCL-CCS and experimental CCS collection, respectively). Experimental CCS values can be browsed on PubChem (<https://pubchem.ncbi.nlm.nih.gov/classification/#hid=106>), while PubChemLite content can be explored at <https://pubchemlite.lcsb.uni.lu>.

3.09.B.T-04 Enhancing Regulatory Environmental Monitoring: Expanding Chemical Domain in HRMS Non-Target Screening

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The assessment of complex chemical mixtures in the environment requires robust and comprehensive quantification methods. Non-target screening (NTS) using liquid or gas chromatography coupled with high-resolution mass spectrometry (LC/GC-HRMS) and cheminformatics workflows provides a

comprehensive overview of chemical exposure, but regulatory monitoring often focuses on a limited number of prioritised compounds. The NORMAN Substance Database (SusDat), with over 100,000 substances of environmental concern, serves as a reference for chemical market emissions. This study, part of the UBA project "Water Monitoring of the Future", integrates data from the German NTS portal and publicly available target data. The NTS portal uses three approaches: database-assisted screening (DBAS) with MS² spectra and retention times for unambiguous identification, suspect screening of known chemicals and their transformation products, and broader non-target analysis to assess chemical exposure in German rivers.

Efforts will focus on expanding the Collective Spectral Library (CSL) to improve overlap between target and NTS data, increase chemical domain coverage and ensure quantification quality. Missing functional groups will be evaluated for ionisation efficiency using electrospray ionisation (ESI) to allow systematic CSL expansion. PubChem fingerprinting and UMAP analysis were used to identify under-represented functional groups, highlighting specific clusters such as phthalates, steroid derivatives and oxazoles. Nitrogen-containing compounds show higher ionisation efficiencies in ESI⁺ and ESI⁻ modes. These findings improve predictions of ionisation efficiency, reduce uncertainties in LC-ESI-HRMS quantification and provide reliable data for regulatory decision-making. By filling gaps in chemical domain, this study establishes a robust framework for evaluating complex chemical mixtures and advancing environmental monitoring.

3.09.B.T-05 Target-Decoy Strategy for Controlling False Discovery Rates in Structure Annotation of Small Organic Molecules in Environmental Non-Targeted Analysis by Computational Mass Spectrometry

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Structure annotation of small molecules by high-resolution mass spectrometry (HRMS) provides insights into the occurrence and fate of unknown organic contaminants in environmental samples. There are now many algorithms based on rule-based, combinatorial, or machine-learning-based methods to rank molecule spectrum matches (MSMs) by correlating molecular fragmentation in tandem mass spectrometry to candidate structures, but such approaches generally have unknown error rates and are thus difficult to use systematically to annotate unknown compounds in practice. Therefore, approaches are needed for estimating false discovery rates (FDR) in MSMs. We have developed an approach for controlling error rates in MSMs using a target-decoy strategy. Target-decoy strategies compare score distributions of candidate molecules (i.e., targets) with those derived from molecules known to be incorrect (i.e., decoys). The target-decoy strategy is common to HRMS-based proteomics, in which reversed peptide sequences are used to construct decoy databases. However, few approaches are available for generating small molecule decoy databases. Here, we describe an approach to generating small molecule structure decoys based on the RDKit cheminformatics software and a custom Python library (SMdecoy). Our approach defines the target database as the set of known molecules for a given molecular formula present in the PubChem database. Next, decoys are generated by mutating and rearranging molecules. Mutation generates new molecules by swapping substructures in candidate molecules with isomeric substructures from the set of all target and decoy molecules. Rearrangement swaps moieties within a molecule to generate new structures. After mutating and rearranging the non-redundant set of target and decoy molecules are combined and the algorithm recurses until a pre-defined number of decoys are generated. Finally, the distribution of MSM scores for target and decoy structures are evaluated based on a pre-defined FDR. Here we will present evaluation of the described target-decoy strategy using MSMs for tandem mass spectra from >400 unique structures using the computational MS tools MetFrag, MAGMa, and CFM-ID. Results demonstrate how FDR control can increase confidence for high-throughput structure annotation of small molecules in environmental samples by HRMS.

3.09.P Future of Suspect and Non-Target Screening to Monitor Emerging Contaminants in the Environment

3.09.P-Tu215 First Steps in the Non-Targeted Identification of Poly- and Perfluorinated Substances using Two-Dimensional Liquid Chromatography Coupled with High-Resolution Tandem Mass Spectrometry (LC×LC-HRMS/MS)

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Poly- and perfluoroalkyl substances (PFAS) represent a real threat due to their environmental persistence and toxicity. The widespread production and various uses of PFAS have resulted in significant

physicochemical variability in the synthesized products and their precursors, leaving many of these chemicals structurally unknown. Therefore, these chemicals require the implementation of complex non-targeted analysis (NTA) workflows using liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) generating high-quality data for their comprehensive identification. However, PFAS chemical space often experiences co-elution of homologs, which can compromise the quality of MS1 and MS2 data, thereby affecting identification confidence.

To this extent, comprehensive two-dimensional LC (LC×LC) provides unparalleled resolving power towards complex PFAS mixtures (e.g., aqueous film-forming foams, AFFFs). Additionally, we hypothesized that the optimized orthogonal separation not only improves PFAS detection in the mass domain but also could increase the quality of tandem spectra (MS/MS) for PFAS identification by resolving chromatographic overlap that occurs in mono-dimensional LC within and among PFAS homologs.

For these reasons, we validated an LC×LC HRMS/MS NTA workflow exploiting mass defect analysis (e.g., remainder of Kendrick mass, RKM) for both the detection and identification of PFAS homolog series (HS) to acquire high-resolution data-dependent (DDA) and independent (DIA) experiments generating high-quality and comprehensive structural MS data.

Results evidenced that LC×LC and RKM prioritization successfully enhanced the quality of PFAS fragmentation patterns allowing for their accurate NTA identification and HS grouping in AFFF complex mixtures. In more details, 12 PFAS HS were successfully categorized, including confirmed compounds and tentative candidates belonging to the HS of perfluoroalkyl carboxylic acids, perfluoroalkyl sulfonic acids, (N-pentafluoro(5)sulfide)-perfluoroalkane sulfonates, N-sulfopropyl dimethylammonio propyl perfluoroalkane sulfonamides, and N-carboxymethyl dimethylammonio propyl perfluoroalkane sulfonamide.

3.09.P-Tu216 Non-Targeted Analysis of PFAS in Drinking and Source Water using Liquid Chromatography High-Resolution Mass Spectrometry and QSRR Retention Time Prediction

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Per- and polyfluoroalkyl substances (PFAS) are categorized within a class that comprises thousands of synthetic chemicals, many of which have been used worldwide to fabricate products resistant to water, heat, and stains since the 1950s. Studies show that exposure to even trace amounts of some PFAS is associated with harmful health effects. The current monitoring program in Canada targets only the most commonly researched PFAS and the number of PFAS characterized in exposure assessments is still relatively small in comparison to the total number registered for commercial use, notwithstanding their transformation products and metabolites in the environment. Non-targeted analysis (NTA) has emerged as a tool that is used to identify and prioritize chemicals for human exposure assessment. While the sample preparation step determines those chemicals that are extracted, it is the data acquisition and analysis stages that ultimately determine chemical identification. Therefore the goals of this study were to develop an NTA method that can provide reproducible identification results. Three data analysis approaches, including FluoroMatch, Compound Discoverer, and FreeStyle were compared with respect to identification reproducibility, all using the same data acquired by liquid chromatography Q-Exactive Orbitrap mass spectrometry. In contrast to FreeStyle, isotopic labelled standards typically cannot be identified with the Fluoromatch and Compound Discoverer methods, meaning the data analysis using these tools is not compatible with the QC protocol in place (which includes spiked internal standards). Therefore, manual searching of isotopic standards, via complementary targeted software such as TraceFinder, is necessary when FluoroMatch/Compound Discoverer are employed. A QSRR retention time model was used to predict the retention times of the tentatively annotated structures to increase identification confidence levels. With this approach, more than ten new PFAS and previously unreported PFAS were identified with a Level 2 degree of confidence in drinking and source water. The results of this study also highlight the importance of reproducible data acquisition. In addition, the practices covered and discussed in this study will benefit and support the advancement of the field of NTA of PFAS.

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3.09.P-Tu217 Detecting PFAS Beyond the Current Regulatory Request: A Comprehensive Overview of the Contamination in Dutch Water by UPHLC-Ion Mobility-HRMS

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The US EPA estimates 15,000 PFAS as manufactured compounds, precursors, and degradation products. With this high number and the lack of reference standards or spectral libraries and plenty of isomers, a systematic and comprehensive monitoring becomes an extremely challenging task. Here, the intention was to sample and analyze wastewater and surface water at 30 sites in the Netherlands to create a map of the PFAS distribution by a non-target, unbiased analysis. UHPLC-HRMS combined with trapped ion mobility (TIMS) was used to identify the PFAS compounds with 4-dimensions of criteria: mass accuracy, isotope pattern fit, MS/MS, CCS (collisional cross sections).

Samples were prepared by SPE according to EPA 1633 with a 2000x preconcentration for surface water and 500x for wastewater. Data acquisition was done with timsTOF Pro 2 (Bruker) in PASEF and bbCID modes. Kendrick mass defect (KMD) filters PFAS compounds from background, based on the fluorine content (repeating CF₂ units). The identification workflow comprised of several steps using library searches, suspect list screening and denovo determination.

In total more than 20,000 features were detected from all effluents and surface water samples of the 30 sampling sites. About 500 potential PFAS candidates were left after the KMD filtering. For the wastewater, in total 188 features could be annotated as PFAS, and for the surface water samples, 137 features could be annotated. The number of detected PFAS varied at the different sampling sites, as expected from their local conditions and infrastructure. A couple of PFAS precursors and degradation products have been found which are not screened yet in any legal directives. These would have been overlooked in a targeted approach. I.e., while the targeted compounds may have been quantified then at actual low levels, the non-targeted degradation products would be present at high levels still posing potential hazard and health risks. An example is H₂-U-PFOS which was identified by denovo determination. This unsaturated PFOS has already been found before in the environment, but a standard is not commercially available yet. Compounds like this one cannot be found with any current target or library search approach. In the presented non-targeted approach, the PFAS analysis is clearly extended beyond those limits since it enables the detection of a virtually unlimited number of PFAS incl. isomers in complex matrices.

3.09.P-Tu218 Identifying Hidden Trends in PFAS Exposure: A Retrospective Suspect Screening Study of High-Resolution Mass Spectrometry Data

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Per- and Polyfluoroalkyl Substances (PFAS) are chemicals known for their water-repellent and flame-retardant properties, widely used in consumer and industrial products.

Water consumption and dietary exposure are the primary pathways for PFAS human exposure. These substances are persistent, prone to accumulate in organisms, and have a half-life in humans of 4 to 8 years, thus raising global health concerns.

Legacy PFAS have been listed as toxic to human and wildlife. Studies have linked PFAS exposure to endocrine disrupting activity and other health issues such as cancer, decreased fertility, abnormal neurodevelopment, and immune system deficiency.

Untargeted lipidomics and metabolomics analysis is increasingly applied to evaluate the impacts of PFAS exposures in epidemiological studies. Despite the high exposure rate, these have mainly focused on metabolic responses to the limited EU-regulated PFAS, likely underestimating the actual extent of exposure to these chemicals.

To tackle this problem, this research aimed at extending proofs of a wider PFAS exposure impacting human health. Retrospective high-resolution mass spectrometry (HRMS) suspect screening analysis allowed us for retrieving PFAS-related spectral patterns in public untargeted lipidomics datasets, expanding the knowledge of PFAS exposure beyond the former targets.

Human lipidomics and metabolomics raw data were sourced from public MS repositories (e.g., Metabolomic Workbench) and previous literature, according to the following criteria: published epidemiological original articles, minimum one event of PFAS detection in study samples, with untargeted metabolomics applied in participants biofluid samples.

A suspect list of commonly detected PFAS and their precursors guided the untargeted screening of the selected HRMS data. Further, this included C₄-C₁₄ perfluoro carboxylic (PFCAs) and sulfonic acids (PFSAAs), ether PFAS, fluorotelomers, and alternative Emerging PFAS precursors.

Negative mode HRMS (both DIA and DDA) datasets were processed using the suspect list, applying the jHRMS toolbox workflow for feature detection, data alignment, clustering, and visualization. The method was validated with recorded PFAS standards spectra. Lastly, investigation of any hidden exposure trends was conducted.

3.09.P-Tu219 Identification of Transformation Products of Pharmaceutical Compounds Generated by Photodegradation by Suspect and Non-Target Screenings

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Pharmaceutical compounds together with their transformation products (TPs) are released into waters from different sources, in particular via the discharge from wastewater treatment plants (WWTPs), and their pseudo-persistence in the aqueous environment may pose potential risks to human health and ecosystems [1]. During wastewater treatment, degradation of pharmaceutical compounds into TPs can take place via biotic and abiotic reactions. Once introduced in the receiving waters, they can be further submitted to solar photodegradation [2]. Moreover, it is now well admitted that some TPs could be more mobile, persistent and/or more toxic than their parent compound [3].

In this context, this study aimed to (1) assess the degradation of some pharmaceutical compounds after simulated solar irradiation (7 model compounds) and irradiation at 254 nm (carbamazepine and atenolol) in spiked pure water and WWTP effluent, and (2) identify the TPs generated by high-resolution mass spectrometry coupled to liquid chromatography (LC-HRMS) in suspect and non-target modes.

Pharmaceutical compounds and some of their known TPs were quantified by target LC-MS/MS by isotopic dilution. TPs were tentatively identified by LC-MS/QTOF using fullscan mode and two DDA acquisition modes: (1) fragmentation of the most abundant entities (DDA abundance), and (2) fragmentation of a list of predefined masses (DDA list; 371 potential PTs identified in the scientific literature and generated in silico [4]). For both simulated solar irradiation and 254 nm irradiation experiments, the workflow was focused on the filtering of the molecular entities present only in the irradiated samples and still present at the end of the experiment (48h or 50% conversion rates), thus corresponding to persistent potential TPs, using the Agilent MPP statistical tool (hierarchical clustering and fold change). Then, TP identification was performed by searching for matches in databases and libraries (DBLs) (Agilent, MassBank, mzCloud and internal library), and an identification level was assigned to each TP [5]. The MetFrag tool [6] and R software were used to assign one or more candidate structures to fragmented entities with no match in the DBLs. All the results will be synthesized and presented to the meeting, also suggesting a list of potential TPs to be monitored in rivers.

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3.09.P-Tu220 Rapid Screening using an Electronic Nose for Evaluating Odor Compound Removal in Reverse Osmosis of Oil Sands Process-Affected Water

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In this study, we propose a monitoring method using an electronic nose to predict and evaluate the performance of the reverse osmosis (RO) according to the pretreatment process in the treatment of oil sands process-affected water (OSPW). The pretreatment process was ultrafiltration (UF) using a ceramic membrane. This study aims to develop and improve analytical methods for the rapid identification of odor-causing substances in treated water OSPW. OSPW contains complex and environmentally persistent dissolved organic mixtures that can be toxic. OSPW always includes several major contaminants, such as naphthenic acids (NA), polycyclic aromatic hydrocarbons (PAH), BTEX (benzene, toluene, ethylbenzene, and xylene), phenols, heavy metals, and ions. These substances have toxicity, and due to the complexity of OSPW, it is challenging to specifically assess the impact of chemical compounds. This study proposes a method to rapidly screen chemical compounds that cause odors in treated OSPW using an electronic nose to quickly identify changes in the treated water. The analytical conditions for the E-nose (HERACLES NEO, Alpha MOS, Toulouse, France) are as follows. Columns used was MXT-1701 (10 m × 0.18 mm i.d., 0.4 µm film thickness). Incubation was conducted at two temperature conditions, 50°C and 70°C, for 20 minutes. The oven temperature program was as follows: hold at 50°C for 25 seconds, raise to 80°C (1°C per second), raise to 250°C (3°C per second), and final hold at 250°C for 21 seconds. The analyzed substances were scored by process using multivariate analysis and utilized to develop a theoretical model for performance prediction in the RO process. For prediction, we used the solution diffusion model (SDM), a widely accepted framework that describes the mechanism by which solutes move across membranes. The data analyzed by the electronic nose were analyzed by applying the SDM that considers

concentration polarization to analyze the experimental results and predict the removal performance under various operating conditions. The prediction model based on the solution-diffusion framework shows a good correlation with experimental data, suggesting its potential usefulness for large-scale system applications. Electronic nose analysis provides a promising solution for monitoring OSPW treated water, as this study confirms that it is a rapid monitoring method for identifying toxic substances in OSPW process treated water

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3.09.P-Tu221 Assessment of Emerging Contaminants in Irrigation Canal Waters: A Suspected Non-Target Screening Approach

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Everyday anthropogenic activities release vast amounts of toxic organic compounds into the environment, including pharmaceuticals, pesticides, industrial chemicals (e.g., per- and polyfluoroalkyl substances), personal care products, and their biotic and abiotic transformation products. Not all of these compounds are regulated under current environmental frameworks as priority contaminants. Instead, many are classified as emerging pollutants-contaminants of emerging concern (CECs)-with limited or no regulatory status. These substances have suspected or fully unknown long-term effects on ecosystems and human health. In Serbia, where wastewater management systems are underdeveloped, the majority of urban and industrial wastewater is directly discharged into surface water without prior treatment. This is particularly concerning in the agricultural region of Vojvodina in northern Serbia, where surface water serves as a critical resource for irrigation.

This study aims to chemically characterize emerging contaminants in the surface water of the Great Bačka Canal, known as one of the most polluted waterways in Europe, employing a suspect screening approach combined with a semi-quantification tool. A total of 40 composite surface water samples were collected along the Canal. Sample preparation involved multi-layer SPE to encompass a wide range of contaminants with varying physicochemical properties. Liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) was used to analyze the extracts. A dedicated data processing software (Compound Discoverer) was employed to detect several thousand chemical features in the water samples. These features were narrowed down to a more focused list of several hundred chemical entities through a stepwise filtering strategy, allowing the identification of compounds with a high level of confidence. The analysis revealed that pharmaceuticals, pesticides, and industrial compounds with multiple applications dominated among the identified contaminants. Sites near urban areas exhibited significantly higher contamination levels, both in terms of the number of detected compounds and their estimated concentrations. The main objective of this work is to advance the understanding of the distribution, diversity, and concentration of emerging contaminants in heavily impacted surface water systems, thereby contributing to future regulatory frameworks and mitigation strategies to protect environmental and human health.

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3.09.P-Tu222 Natural Wetlands: Challenging the Conventional Perspective on Wastewater Treatment Processes

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Current global declines in freshwater quality and quantity are pivotal issues being worsened by rising human populations, heightening agricultural, industrial, and urban water demands. These anthropogenic pressures increase the exposure of natural ecosystems to organic contaminants via wastewater, posing risks to both humans and the environment. While effective at remediating chemical contamination, wastewater treatment plants (WWTPs) are costly and in many cases require unrealistic development to mitigate the release of all types of chemicals from entering freshwater sources. Wetlands offer a promising, low-cost alternative for terminal wastewater treatment, being termed nature's kidneys. This

study examines the ability of the Frank Lake (FL), AB, Canada, an agro-industrial and municipal effluent-driven wetland complex to remediate organic contaminants, while assessing the potential toxicity of the combined effluent. To begin addressing these points, non-targeted analyses were performed via ultra-high performance liquid chromatography high resolution mass spectrometry to identify and semi-quantify organic chemicals in both sediment and water extracts. The toxicological properties of all annotated compounds were assessed by the USEPA ToxCast program. In vitro and 21-day in vivo examinations of endocrine disruption to the T47D-KBluc human breast cancer cell line using estrogen receptor assays, and Fathead Minnows (*Pimephales promelas*) were also conducted. To date, 773 unique compounds were identified in FL samples; 202 of which exhibit toxicological effects. The relative abundance of pharmaceuticals, one of the most prevalent use classes of chemicals in this mixture, was shown to decrease as water flows through the wetland; highlighting its potential as a remedial alternative to terminal WWTP processes. Similarly, the contamination at the combined site of municipal and agro-industrial effluent release resulted in endocrine-disrupting impacts including estrogen receptor transactivation during in vitro assessments, decreased male *P. promelas* fertility, and increased tubercle scores, with reduced to undetectable effects downstream. The results herein showcase the potential for natural wetlands to minimize contaminant loads that enter downstream water supplies, and thus their related toxicological impacts on freshwater ecosystems; providing an alternative solution towards sustainable water pollution management.

3.09.P-Tu223 Prioritizing Organic Toxicants in Hydraulic Fracturing Flowback and Produced Water from Shale Gas Sites using Integrative Effect-Directed Analysis and Non-Target Screening
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The use of hydraulic fracturing in shale gas exploitation has generated substantial amount of flowback and produced water (FPW), and ecological risk of these highly complex chemical mixtures has raised worldwide concern. Herein, an integrative effect-directed analysis (EDA) and nontarget screening (NTS) workflow was developed to identify and prioritize main toxicants in treated FPW (T-FPW). The workflow included sample extraction and fractionation, zebrafish embryo toxicity tests, target and nontarget chemical analyses, and toxicity prioritization and confirmation using toxicological priority index (ToxPi). Results showed that less hydrophobic compounds which were used in fracturing fluid and their degradation products were the potentially high-risk toxicants in T-FPW. Thirty-nine target compounds identified in toxic fraction explained 4.82% of the mortality. Additional 584 nontarget contaminants were annotated by NTS. Risk prioritization was achieved for 470 identified contaminants with ecotoxicity data available using a ToxPi method. Six nontarget toxicants were identified with higher ecological risks than all target contaminants, and their presence in FPW were confirmed using reference standards. A principal component analysis of NTS features revealed that EDA fractionation reduced mixture complexity and focused toxicant screening, which significantly improved NTS efficiency, highlighting advantages of integrative EDA and NTS for mixture risk assessment.

3.09.P-Tu224 Combining Advanced Analytical and Effect-Based Approaches for Characterization of Wastewater

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Over the past 15 years, advances in analytical screening techniques, particularly with suspect and non-target screening, have significantly improved the characterization of wastewater. These approaches allowed the identification of various contaminants, highlighting the potential of wastewater-based epidemiology to serve as an early warning system for environmental hazards. However, many compounds in this complex matrix remain undetected and uncharacterized, thereby presenting potential threats to the environmental ecosystems. To address these challenges, we implemented an integrative approach combining advanced analytical techniques, suspect and non-target screening, with effect-based in vitro assays to comprehensively characterize and assess contaminants present in wastewater. Samples were collected from seven locations within Örebro, representing residential, industrial, and mixed-use areas, with global influent and effluents samples. Sampling was conducted in 2023, in May and September. Two extractions protocols were used on the collected samples, generating extracts for an apolar and a polar fraction. Each fraction was split for organic contaminants analysis, using liquid and gas chromatography coupled with high resolution mass spectrometry (LC- and GC-HRMS), and effect-based in vitro assessment, consisting of the aryl hydrocarbon receptor (AhR; DR CALUX), androgen receptor antagonism (AR CALUX) as well as estrogen receptor ? (ER? CALUX) activities. Our analysis revealed site-specific differences in contamination profiles, with pharmaceuticals such as lidocaine being

predominantly detected in the residential areas, whereas plasticizers such as triphenyl phosphate were more prevalent in the industrial samples. The results from the effect-based methods showed variability in receptor activity between the different fractions and sampling areas. Specifically regarding ER α activity, apolar extracts displayed higher bioactivity compared to the polar extracts, with all samples being bioactive in September and bioequivalent concentrations reaching up to 31.9 μ g/mL in the residential area. Further research is necessary to elucidate the relationship existing between the detection of chemical contaminants and their biological effects. This study demonstrates the value of integrating innovative analytical strategies with effect-based methods for evaluating wastewater, providing comprehensive insights for improving environmental monitoring strategies in the future.

3.09.P-Tu225 An Extensive Pollutant Characterization Method for Organic Fertilizers Combining Accelerated Solvent Extraction, Chemical Analysis and Cell-based Bioassays

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Increasing the circularity of nutrients essential for crop growth is a vital part of the Circular Economy Action Plan designed by the European Union. In Sweden, sewage sludge, cow manure, and fermented food waste are the prevalent types of organic fertilizers (OF), mainly acting as fertilizers for phosphorus and organic matter.

However, the presence of a diverse range of emerging micropollutants has raised concerns around the use of OF. While optimized methods for extraction and chemical analysis exist for individual pollutant groups, there is a pressing need for a more comprehensive method to streamline the pollution assessment of such fertilizers.

This study aims to fill this gap by combining an Accelerated Solvent Extraction (ASE) method with GC- and LC-HRMS analyses as well as CALUX ® bioassays. Combining chemical analysis with bioassays gives insight into pollutant groups present as well as resulting mixture and toxicity effects. Sewage sludge samples were acquired from 9 wastewater treatment plants with differently sized catchment areas (cities, towns, rural) across Sweden. Additionally, 5 cow manure samples and 5 food waste fermentate samples were included in the study.

Extraction was performed both with apolar (n-hexane and dichloromethane mix) and polar (methanol) solvents. Using a spike-addition approach, recoveries of apolar contaminant groups such as polychlorinated biphenyls, dioxins and furans, polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons, and siloxanes were tested. The recoveries for polar contaminant groups, including per- and polyfluoroalkyl substances, phthalates, bisphenols, phosphorus flame retardants and a mix representing widely used pharmaceutical classes were also evaluated. Unspiked extracts were assessed with an array of CALUX ® bioassays, testing for estrogenic, anti-androgenic, xenobiotic effects as well as for oxidative stress (ER α , anti-AR, DR, Nrf2 CALUX ®).

Our results show that performing both an apolar and polar extraction is necessary to capture the different pollutant groups present. For example, the bio-EEQ values differed greatly between the apolar and polar extracts in the ER assay but to a much lesser extent in the DR assay (bio-TEQ values).

ASE combined with GC- and LC-HRMS analysis seems to be promising to extract and characterize a wide range of pollutants present in OF. Bioassays like ER α , anti-AR, DR, Nrf2 CALUX ® can give further insights into extraction efficiencies of bioactive compound classes.

3.09.P-Tu226 Identification of Major AhR and ER Agonists in Freshwater Fish from the Gapcheon River in Korea Using Effect-Directed Analysis and Nontarget Screening

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This study aimed to identify major aryl hydrocarbon receptor (AhR) and estrogen receptor (ER) agonists in freshwater fish collected near the sewage treatment plant (STP) outfall in the Gapcheon River using effect-directed analysis (EDA) and nontarget screening (NTS). Target species included common carp (*Cyprinus carpio*, S1), crucian carp (*Carassius carassius*, S2), Far Eastern catfish (*Silurus asotus*, S3), barbel steed (*Hemibarbus labeo*, S4), and sky gager (*Erythroculter erythropterus*, S5). The accurate trophic positions of fish, calculated from $\delta^{15}\text{N}$ values of amino acids, were 2.2 for S1 and S2, 3.0 for S3, and 3.2 for S4 and S5. The H4IIE-luc bioassay revealed elevated AhR-mediated potencies in the mid-polar fractions of S2 egg, S2 muscle, S3 fillet, and S5 liver. The T47D-kbluc bioassay identified significant ER-mediated activities in the polar fractions of the S2 egg and S2 muscle. Twenty-one targeted AhR agonists,

including 7 traditional polycyclic aromatic hydrocarbons (PAHs) and 14 emerging PAHs, accounted for 49%, 94%, 83%, and 20% of AhR-mediated potencies in the S2 egg, S2 muscle, S3 fillet and S5 liver, respectively. Among these, benzo[b]anthracene was the dominant contributor, explaining 18% to 68% (mean = 44%) of AhR-mediated potencies. In contrast, 4 known ER agonists, such as estrone, 17 β -estradiol (E2), estriol, and 17 β -ethinylestradiol, explained only 7.7% and 18% of ER-mediated potencies in polar fractions of S2 eggs and muscle, respectively, with E2 contributing 7.6% in eggs and 17% in muscle. To identify unknown ER agonists, NTS using LC-QTOFMS was conducted on the highly potent polar fraction of the S2 eggs. A four-tier selection criteria identified 15 and 12 candidates in positive and negative ionization modes, respectively. Toxicological confirmation using the T47D-kbluc bioassay revealed significant ER efficacies for mestanolone, mesterolone, and methandrostenolone in positive mode, and alfatriol, 2-hydroxyestradiol, 17-epiestriol, and epiestriol in negative mode. The inclusion of these novel ER agonists increased the explanation rates of ER-mediated potencies to 27% in eggs and 21% in muscle, with 17-epiestriol (18%) dominating in eggs and epiestriol (2.7%) in muscle. The findings highlight the need for further studies on the sources of these AhR and ER agonists, particularly their association with STP effluents, environmental pathways, ecotoxicity, and accumulation across trophic levels in aquatic organisms.

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3.09.P-Tu227 Efficient Identification of Chemical Indicators for Enhanced Pollution Management in Industrialized Coastal Regions

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Marine sediments are crucial indicators of anthropogenic pollution, yet traditional analytical approaches often fall short in capturing the full complexity of non-targeted contaminants in intensively industrialized coastal environments. To address these challenges, this study introduces a refined workflow and software tool tailored for non-target screening (NTS) using a gas chromatography coupled to a time-of-flight mass spectrometry (GC-TOF/MS). By incorporating retention index (RI) filtering, the developed tool effectively improved data processing and enhanced the identification of diagnostic chemical indicators in marine sediments.

Sediment samples from Okpo and Onsan Bays, which are close to big-sized industrialized complexes, were analyzed using the developed software including the automated extraction process of reliable chemical features with reduction of low-quality data. The workflow narrowed over 2,000 detected features to 60 diagnostic compounds through systematic filtering and validation processes. The software demonstrated strong validation accuracy (87%) and significantly reduced the manual effort required for data analysis, offering practical improvements to current NTS workflows. Key compounds identified include cyclic siloxanes, n-alkanes, and polycyclic aromatic hydrocarbons (PAHs), which were linked to distinct pollution sources via Partial Least Squares-Discriminant Analysis (PLS-DA). The PLS-DA model exhibited robust predictive performance ($Q^2 = 0.938$) and statistical significance ($p < 0.0005$), demonstrating the tool's reliability for diagnostic applications.

This study highlights the potential of the software to improve environmental monitoring practices, offering a scalable and efficient tool for analyzing complex contamination profiles. By refining NTS workflows, the developed methodology presents a practical approach for tracing pollution sources, with meaningful implications for enhancing pollution management strategies and informing regulatory frameworks in industrial coastal areas. This provides a foundation for additional studies to expand the application of this tool across diverse environments and contamination scenarios, contributing to broader efforts in environmental conservation.

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3.09.P-Tu228 Non-Targeted Microplastic Leachate Analysis Using Dual Electron Ionization/Chemical Ionization Gas Chromatography-High Resolution Mass Spectrometry

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The production of synthetic polymers has created an environmental threat due to plastic pollution. Microplastics; plastic particles under 5 millimeters, degrade chemically and physically in the environment, forming secondary microplastics and low molecular weight compounds that can leach into ecosystems. Gas chromatography (GC) combined with electron ionization (EI) mass spectrometry (MS) has been used for analyzing volatile and semi-volatile compounds, with fragmentation patterns easily matched to databases such as NIST. However, identifying the molecular formula of the intact compound is challenging due to the extensive EI fragmentation. Chemical ionization (CI) preserves intact molecular ions, however, to provide both, EI fragment and exact molecular mass information of the sample, two separate GC runs are usually required. This approach increases analysis time, demands for complex data alignment, and poses the risk of sample degradation/changes between the two individual runs. To address these limitations, a dual-source setup capable of obtaining EI and CI information within a single chromatographic run was used in this work.

Samples were obtained from NORMAN network's joint program activity Microplastics-leaching of additives and non-intentionally added substances. They were extracted using 1-propanol as an extraction solvent and analyzed using a consistent GC method across high-resolution and low-resolution mass spectrometry setups. The employed ecTOF system (Bruker, Bremen, Germany) allowed quasi-simultaneous EI and CI data acquisition. Data processing included peak picking, deconvolution, and alignment, with multivariate analysis such as orthogonal partial least squares discriminant analysis, identifying significant variables. Features were prioritized based on Variable Importance in Projection ranking, with retention indices and mass spectra compared against NIST spectral databases and open-source libraries.

Comparative analysis of microplastic leachates across GC-MS platforms showed differences in sensitivity and confidence levels in compound identification. This study highlights the advantages of CI spectra, particularly for compound classes like hydrocarbons, where fragmentation patterns are similar regardless of chain length and molecular ion data is missing. The number of detected compounds and confidence levels are compared, and the importance of high-resolution EI spectral libraries is demonstrated, showcasing specific examples.

3.09.P-Tu229 Development of a GC-HRMS Spectral Database for Photolytic Degradation Byproducts of Common Organic Pollutants

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Photodegradation is a significant degradation mechanism for many environmental organic contaminants, both indoors and outdoors. Many of these contaminants are persistent and toxic, but the exact degradation processes for many of these are not well understood. It is crucial to monitor and study commonly found environmental contaminants and identify their degradation products. However, the lack of standards makes identification difficult. Some of the most common mass spectral libraries are for gas chromatography coupled with mass spectrometry (GC-MS) that are recorded at low resolution. However, this makes it challenging to identify compounds with spectral peaks that differ only slightly in their mass-to-charge ratio (m/z). However, new GC-MS instruments with high mass resolution are more and more common.

The aim of this project was to determine potential degradation products from various compounds commonly found in indoor environments and add these findings to a spectral library for high-resolution mass spectrometry (HRMS). The study involved exposing standards to ultraviolet (UV) light and performing chemical analysis using GC-HRMS. The data was processed using MS-DIAL to identify potential products, and a workflow to generate spectral library was developed to enable suspect screening in environmental samples. Many compounds did not show any degradation under the experimental conditions. This showcases some challenges in the current experimental setup. However, four potential degradation products were found: penta-, tetra-, and tribromobenzene from hexabromobenzene (HBB), and one potential degradation product from 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB).

3.09.P-Tu230 High-Resolution Mass Spectrometry Screening of Quaternary Ammonium Compounds (QACs) in Dust from Homes and Various Micro-environments in South China

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Despite their ubiquitous use, information regarding the presence of Quaternary ammonium compounds (QACs) in various micro-environments remains scarce and only a small subset of QACs have been monitored using targeted chemical analysis. In this study, a total of 111 dust samples were collected in homes and various public settings from South China during the COVID-19 pandemic, and were analyzed for traditional and emerging QACs using high-resolution mass spectrometry. The total traditional QAC concentrations in residential dust (traditional QAC, sum of 18 traditional QACs) ranged from 13.8 150

µg/g with a median concentration of 42.2 µg/g. Twenty-eight emerging QACs were identified in these samples and the composition of emerging QAC (sum of emerging QACs) to QAC (sum of traditional and emerging QACs) ranged from 19 to 42% across various micro-environments, indicating the widespread existence of emerging QACs in indoor environments. Additionally, dust samples from cinemas exhibited higher QACs concentrations compared to homes (medians 65.9 vs. 58.3 µg/g, respectively), indicating heavier emission sources of QACs in these places. Interestingly, significantly higher QAC concentrations were observed in dust from the rooms with carpets than those without (medians 65.6 vs. 32.6 µg/g, $p < 0.05$, respectively). Overall, this study shed light on the ubiquitous occurrence of QACs in indoor environments from South China.

3.09.P-Tu231 Development and Validation of an Open Access High Resolution Mass Spectral Library for Detection of Food Toxicants

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Food represent one of the most difficult sample in trace analysis. Due to the high chemical complexity (sugars, lipids, amino acids, etc.) and to the high concentration of major components (such as fatty acids in cereals), searching for low-abundance contaminants or residues in food can be considered a very complex and challenging task.

Nowadays, quali-quantitative analysis of food contaminants mainly relies on the use of hyphenated techniques, such as liquid or gas chromatography coupled to low or high resolution mass spectrometry (namely LRMS and HRMS). The combination with LRMS is often considered the best choice for the targeted strategies. Although this approach is a highly sensitive and selective, it requires prior knowledge of the analytes of interest (e.g., their precursor and product ions), and is not suited for retrospective analysis of extra compounds.

Contrarily, HRMS is the state-of-the-art detection technique for semi-targeted or non-targeted analysis and can be considered as a powerful tool for the detection and identification of known and unknown chemicals. The use of high resolution tandem mass spectra library (HRMS2) helps in the identification of compounds. Nevertheless, most of the them cover natural products and only a few contaminants.

Moreover, most of the HRMS2 libraries repositories focus on total number of spectra more than on the quality, for example combining data acquired from different instruments and at nominal and accurate mass.

The aim of the study was to develop and validate a HRMS2 library containing well-known food hazard chemicals, among which veterinary drugs, contaminants, pesticides and natural toxins (including metabolites). The library was built acquiring standards in solvent using ultra high-pressure liquid chromatography (UHPLC) coupled to an Orbitrap IQ-X Tribrid, with positive ESI as the ionization interface. Each compound was acquired using 7 different collision energies generating more than 7,000 mass spectra in total. All the information related to the strategies used for built the library are included here.

Overall, integrating this library into routine testing workflows has the potential to improve food safety monitoring and support more robust regulatory oversight, ultimately benefiting public health and safety.

3.09.P-Tu232 Analysis of Pesticide Residue Using High-Resolution Mass Spectrometry in Korea Herbal Medicines

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As rapid industrialization and people's living standards have improved, people have become more interested in quality and safety in food. In particular, direct hazards that impede food safety can be broadly divided into contaminants and residual substances. This has always been present in food production sites, processing industry, distribution processes, or sales processes, and is known to contaminate food in various ways. To solve this problem, developed countries have been investing large amounts of budget to develop and operate hygiene programs such as HACCP for a long time, and Korea has also been operating them under the management and supervision of MFDS. In addition, we are continuously developing residual material analysis methods and residual material testing programs and utilizing them in the field. Among these, looking at the development status of Korea's simultaneous multi-component residue analysis methods, MFDS about 510 types of pesticides and NAQS about 463 types of pesticides is conducting safety management of pesticide residues in agricultural products. Testing was conducted using HPLC and GC as analysis equipment for safety management of pesticides residues, but recently, testing has been conducted using MS/MS. MS/MS has the advantage of high selectivity and sensitivity, but has disadvantage of being able to analyze only the compounds we designate. However, HRMS has the great advantage of not only being able to identify the exact compound due to its high resolution, but also being

able to track when the problem began by extraction specific ions from the total ion chromatogram. Therefore, pesticide residue analysis using HRMS can track not only pesticide residues but also other contaminants or residues, and has excellent selectivity, making it possible to accurately determine compounds. If an analysis method using HRMS is developed, it can be used to determine the compounds in food and it is expected that safety management of contaminants and residual substances will be further strengthened

3.09.P-Tu233 Development of an Optimized Workflow for Suspect Screening of Persistent, Mobile, and Toxic (PMT) Substances in Circular Food Systems

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The transition to circular food systems introduces food safety challenges arising from chemical contaminants with persistent, mobile, and toxic (PMT) properties, including pesticides, pharmaceuticals, industrial chemicals, and environmental pollutants. These substances, numbering over 15,000 in international databases, are particularly concerning in recycled materials like sewage sludge. Conventional methods for PMT identification, reliant on reference standards, are limited in scope and applicability to complex matrices. To address these gaps, we developed a high-resolution mass spectrometry (HRMS)-based workflow for comprehensive suspect screening of PMTs across a broad polarity spectrum in sewage sludge.

The workflow includes a UHPLC-HRMS analysis, employing Data-Dependent (DDA) and Data-Independent Acquisition (DIA) for enhanced scan data collection, alongside a spectral library and inclusion lists derived from the NORMAN database. Two sample preparation methods, SPE-based and QuEChERS-based, were evaluated, with the latter optimized for sensitivity and reproducibility. Suspect PMTs were screened using Compound Discoverer software and matched against the NORMAN database to identify novel compounds. Targeted analysis of reference standards enabled accurate quantification, while suspect screening expanded the detection scope. Testing on 22 sewage sludge samples revealed known and suspect PMTs across diverse sources, including municipal, plant-based, and animal-based residuals.

This workflow provides a robust, transferable method for identifying PMTs in complex matrices without dependence on reference standards. By bridging critical analytical gaps, it supports improved monitoring and risk assessment of chemical contaminants in circular food systems.

3.09.P-Tu234 Combining Retention Factor and Retention Index: A New Approach for Enhancing Retention Prediction Using Chemometrics

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Liquid chromatography is among the most widely employed techniques across numerous analytical fields, including the analysis of pharmaceuticals, environmental samples, proteins, and polymers. Unfortunately, method development in liquid chromatography can be very time-consuming and challenging due to the high number of variables that need to be optimized such as the mobile and stationary phase selection, the establishment of a fitting solvent gradient, choosing an appropriate flow rate, etc. This is especially true for complex samples. Therefore, efficient optimization techniques such as retention modeling are needed to support the method development.

In the past, two very useful parameters, namely retention factor and retention index, were explored in order to develop an accurate model for retention prediction. A novel machine learning algorithm, for example, was developed to predict the retention indices for structurally unknown chemicals based on cumulative neutral losses. On the other hand, multiple retention models exist that are based on the retention factor such as the linear solvent strength model or the Neue-Kuss model. While the retention factor is directly influenced by the chromatographic conditions it requires multiple measurements to determine the retention model accurately. The retention index depends on the relative retention compared to that of reference substances and can be used to more easily compare different systems and conditions. As of yet, no attempt has been made to combine the retention factor and the retention index for additional information to train the machine learning model. Although the results of individual models look promising, combining both retention parameters could give new insights and lead to more accurate predictions. Therefore, in this project, the retention factor and retention index will be combined into a new parameter, namely the retention score. This new parameter will allow accurate prediction of substances and give a first estimate of whether two substances can be separated by using a certain method.

3.09.P-Tu235 Refining Molecular Networks: The Power of Distance Metrics in Spectral Differentiation

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Molecular networks are a visualization that compares every molecule to one another based on their mass spectra, enabling the clustering of similar species based on a similarity score and a specific threshold. These networks are utilized in a wide spectrum of bio and chemistry fields, including the likes of environmental sciences, biomedical, and forensics, to name a few. Since mass spectra are directly related to molecular structures, similar structures will be linked within the network, enabling the identification and exploration of structural similarities and, therefore, related molecules.

For this study, we utilized electrospray ionization (ESI) MS2 spectra, which enables the comparison of molecule fragments within the networks. ESI spectra can deviate between instruments and experimental conditions, making the matter more complex that is observed experimentally and recorded in mass spectrometry databases such as MassBank EU, GNPS (The Global Natural Product Social Molecular Networking), MoNa (MassBank of North America), or NIST (National Institute of Standards and Technology).

Algorithms used to build such networks tend to utilize the cosine score for similarity testing. However, there are a vast majority of different metrics to calculate such scores, i.e., Euclidian Distance, Jaccard Index, Manhattan, Minkowski, and Sørensen-Dice coefficient. We investigated these five different similarity algorithms and their influence on the network thereof. Since different values are obtained from each algorithm, we observed direct influence within the molecular networks. Utilizing different visualization strategies in combination with the varying similarity algorithms was also found to be significant for the formation of molecular networks.

The utilized data contained multiple spectra for each molecule with, i.e., different ionization energies. Therefore, the results were further investigated and connected to the conditions used for the data acquisition. The spectra not used to build the model were used as a test set, which allowed us to evaluate how these similarity metrics influence the level of confidence during the chemical identification via spectral matching.

3.10.A Analysis, Assessment and Management of Contaminants of Emerging Concern and Their Transformation Products

3.10.A.T-01 Keep it Cool in Your Lab! Why "Cold" OECD TG 309 Degradation Tests are the New Hot Trend for Assessing Chemical Persistence

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The threat to drinking water resources posed by persistent and mobile substances has been scientifically highlighted for decades and the occurrence of polar chemicals in the aquatic environment has been known since the 1980s. ECHA recommends that the OECD TG 309 degradation test should be performed first as a standard. The goal of the OECD TG 309 degradation test is to demonstrate intrinsic aerobic biodegradability and to derive a valid half-life for aerobic biodegradation (degradation half-life, DegT50) in surface water.

When assessing whether the result is valid two cases must be distinguished: Case 1: A pronounced and significant decrease in the concentration of the test substance in the aqueous phase is observed during the test. This case most likely needs a 14C-labeling of the test substance for a valid test result. Case 2: No pronounced and significant decrease in the concentration of the test substance in the aqueous phase is observed during the test. For a valid test result it is only necessary to demonstrate that a sufficiently high aerobic biodegradation potential (i. e. a vital inoculum) was present in the test system. This is ensured using a reference substance. In case 2, a valid result of the degradation test according to OECD TG 309 can always be obtained without 14C-labeling of the test substance. Case 2 is expected especially for persistent and mobile chemicals.

Criteria were defined to assess if a test substance is suitable for a "cold" degradation test according to OECD TG 309. The criteria were applied to assess a list of 1250 known water contaminants. The list of 832 contaminants prioritized for a "cold" degradation testing according to OECD TG 309 has been divided into two priority levels: contaminants that have already been detected in drinking water, groundwater, raw water or bank filtrate (category A, 422 contaminants) and contaminants that have only been detected in wastewater treatment plant effluent and/or surface water (category B, 410 contaminants).

23 Recommendations for a simple, cost-effective and efficient implementation of a valid "cold" degradation test according to OECD TG 309 were developed. The recommendations apply only to those test substances for which no pronounced and significant decrease in concentration in the aqueous phase is

expected during the test. Our work will support drinking water suppliers and water monitoring authorities to carry out OECD TG 309 degradation tests in their own analytical laboratories

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3.10.A.T-02 Sulfamethoxazole Transformation by Heat-Activated Persulfate: Linking Transformation Products Patterns with Compound-Specific Isotope Analysis

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Sulfamethoxazole (SMX) is a frequently detected sulfonamide antibiotic in surface and groundwater, raising environmental concerns about its fate. Oxidative treatments, such as persulfate application, are commonly used for micropollutant removal. To investigate and differentiate SMX transformation by various radicals from other SMX dissipation processes using multi-element compound specific isotope and transformation products (TP) analysis, SMX transformation experiments were conducted using heat-activated persulfate at pH 3, 7, and 10. SMX hydroxylamine (TP269a) and TP178 were identified as the dominant transformation products across all pH levels. The exclusive formation of 4-nitroso-SMX, 4-nitro-SMX, and TP518 at pH 3 highlighted the role of SO₄^{•-} in attacking the amino group. At pH 7 and pH 10, 3A5MI emerged as the dominant TP. Normal carbon isotope fractionation (δ¹³C from 1.9 to 2.3 ‰), with consistent isotopic values across pH levels, was attributed to the formation of TP178, which involves C-S bond cleavage. An inverse nitrogen isotope fractionation at pH 3 (δ¹⁵N = +0.68 ± 0.11 ‰) was linked to SO₄^{•-}-induced single-electron transfer, leading to the formation of N-centered SMX radicals. Conversely, normal nitrogen isotope fractionation at pH 10 (δ¹⁵N = -0.27 ± 0.04 ‰), was associated with multiple bond cleavages, including N-H bond cleavage initiated by H abstraction through HO[•] and N-S bond cleavage leading to the formation of 3A5MI. The inverse nitrogen isotope fractionation observed at pH 7 indicated that the dominant pathway involved SO₄^{•-} reactions, accounting for 76% of the overall transformation. Overall, the results highlight the potential of CSIA to elucidate SMX persulfate oxidation pathways and evaluating the natural attenuation of SMX through radical reactions in aquatic systems.

3.10.A.T-03 Towards Predicting Persistent Transformation Products in Different Environments

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Transformation products (TPs) are gaining attention in the scientific community and chemical regulation, in particular if they are persistent, mobile, or toxic. Observed TPs and degradation rates differ between environments, highlighting the necessity to better understand environment-specific biodegradation processes. For regulation dealing with long lists of chemicals and for industry trying to integrate Safe-and-Sustainable-by-Design principles early in the development of new chemicals, tools for fast and reliable prediction of biodegradation processes are direly needed. We believe that in silico identification of persistent TPs in a given environment from chemical structure alone can be achieved by combining pathway- with persistence prediction.

The pathway prediction engine of the online platform envPath is commonly applied to generate suspect lists for analytical screening of TPs. We have recently increased the scope of envPath databases by adding biodegradation data on micropollutants in activated sludge and water-sediment systems. The envPath database provides training data for new machine learning models developed in our group that can predict persistence endpoints (e.g., primary biotransformation half-lives, breakthrough in wastewater treatment plants), of complex chemicals and provide a reliable uncertainty estimate for each prediction. Here, we apply a model trained on soil half-lives of pesticides to predict persistence of experimentally observed transformation products in soil and activated sludge for which no kinetic data is available. We show that the model can point towards TPs that, although classified as minor, may be persistent in soil. We believe that the combination of data collection efforts and new approaches to persistence prediction will help achieving a comprehensive understanding of biodegradation processes, and pave the way towards the integrated, environment-specific prediction of persistent TPs.

3.10.A.T-04 Identifying Potentially Hazardous Transformation Products from Compounds on the Global Chemical Inventory

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Identifying persistent, mobile, and toxic (PMT) chemicals and their transformation products (TPs) is essential for assessing environmental and health risks. However, public data on TPs is limited: of over 140,000 compounds in the global inventory, only 0.9% have TP data in PubChem. Due to high computational time and numbers, on the fly predictions often cover only 1-2 generations. A comprehensive database with multi-generation TPs is therefore needed to track potentially harmful TPs from longer reaction pathways.

As a proof of concept, 5 generations of environmental TPs were predicted for 321 pesticides using the "env" and abio model in BioTransformer 4.0 (version 2024-11-06). The results were stored in a Neo4j database for fast querying via a web interface that was built with Python, JavaScript, Cypher, and HTML. Dead-end TPs (TPs to which no reaction rules apply) were identified and assessed for mobility, persistence, and toxicity using OPERA predictions.

Starting from 321 initial pesticides, the BioTransformer predictions yielded 752,262 predicted TPs from 1,794,271 unique reactions. However, only 280 of these TPs are predicted to be dead-end products, i.e., TPs with potentially greater environmental persistence concerns. Out of the 280 predicted dead-end TPs, 201 were predicted to be mobile, of which 172 were also very mobile based on the EU CLP criteria. As for persistence, only 149 of the TPs fell inside the model applicability domain, of which 46 were predicted to not be readily biodegradable. One of these vPvM TPs, pyridine N-oxide, had 6 different precursors among the 321 input compounds, all of which shared the pyridine substructure. Thus, similar patterns may be useful for grouping efforts in combination with experimental confirmation of the TPs and their properties. Only 11,198 (0.15%) of the TPs in the GCI-TPs database were found in the PubChem database based on a full InChIKey match. This is concerning from a non-target analysis point of view as structure databases such as PubChem are typically used for annotation. This shows that GCI-TPs can potentially help fill an important gap in annotation workflows. The GCI-TPs web interface has several different search options to facilitate querying, including finding all precursors or TPs of a compound, reaction display and batch searches. The predictions mentioned above are now being scaled to the complete 140,000 compound global chemical inventory.

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3.10.P-Th148 Seasonal Variation of Contaminants of Emerging Concern in Swedish Landfill Leachate and its Recipient: A Case Study

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Landfill leachate is an important point source of contaminants of emerging concerns (CECs) in fresh water systems. There are over 350 active landfills in Sweden, producing 8 ~12 million m³ of leachate each year. This study conducted non-target screening (NTS) and suspect screening (SS) using ultra high-performance liquid chromatography coupled with high resolution mass spectrometry (UHPLC-HRMS) to identify the CECs in Swedish landfill leachate. The aim of this research is to map the occurrence of CECs in Swedish landfill leachate and provide insights into their seasonal changes.

In our study, 24-hour composite samples of untreated, treated leachate and recipient upstream, and downstream (named UL, TL, RU, and RD) were collected from a municipal landfill in Sweden in September 2023, January, March, and June 2024 by time-integrated autosamplers. Extraction was performed on multi-layer solid phase extraction (SPE) cartridges in order to capture a wide spectrum of CECs. Preliminarily, 74 CECs were identified in all the landfill leachate samples, including 35 industrial chemicals, 21 pharmaceuticals, 10 PFASs, and 9 pesticides. Winter had the highest number of detected CECs and the highest detection frequency among all seasons. 49 CECs were detected in UL, TL, and RD across all seasons, and 16 of them were also detected in RU in either or all seasons. Some industrial chemicals (e.g., tripropyl phosphate and dibutyl phthalate) showed lower detection frequencies in warmer seasons (summer and/or autumn), while some pharmaceuticals, such as bicalutamide, losartan, and lamotrigine, had lower detection frequency in spring and summer. Possible reasons for the seasonal change in the occurrence of CECs can be: (1) increased removal efficiency at higher temperature; (2) the amount of produced leachate and the concentration of CECs can be influenced by precipitation; (3) the reception of external waste may vary depending on time. These findings highlight the need for seasonal

monitoring of CECs and adjustments of leachate treatment strategies to manage the diverse presence of CECs in landfill leachate throughout the year.

3.10.P-Th149 Synthetic Musk: From Wastewaters to Surface Water Ecosystems

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Synthetic musks are compounds incorporated in detergents, perfumes, and house-cleaning products. Within them there are Galaxolide, its metabolite Galaxolidone, Tonalide, Celestolide and Phantolide. They are released every day in large amounts into sewage systems and discharged in aquatic ecosystems through wastewater treatment plant (WWTP) effluents. The prolonged exposure of aquatic environments to the continuous discharge of PMFs is raising many concerns about their possible chronic toxicity and future impacts. Data regarding PMFs in Italy are very scarce even if this is the EU-Member State with the highest consumption of these chemicals. In this study, we sampled wastewater in four different stations of a conventional WWTP with activated sludges to understand fate and behaviour of PMFs during treatments. PMFs were measured in all stations up to the $\mu\text{g/L}$ order of magnitude. Biotransformation and adsorption were the two processes driving the fate of these contaminants during treatments. High and stable musk concentrations were measured in activated sludges. Only a slight reduction of the main PMFs, Galaxolide (-20%) and Tonalide (-30%), was evidenced during treatments, together with the increase of the Galaxolide metabolite (+70%). This leads to discharges of these compounds in receiving water bodies which can be degraded by WWTP effluents. PMF presence was then assessed in an anthropized surface water ecosystem, Lake Maggiore, considering both water and sediments of the main tributaries to investigate concentrations entering the lake but also two freshwater bivalves to assess possible bioaccumulation. In waters, characterized also in terms of pH, conductivity, alkalinity, major ions and nutrients, PMFs were detected up to hundreds of ng/L especially in small rivers in which the highest values of N and P were measured. Sediment analyses followed the same degree of pollution with concentration peaks detected during summer seasons. Molluscs analysis reflected the degree of anthropic pressure present on the lake shores, highlighting two different bioaccumulation patterns in the two considered species. In all matrices, Galaxolide, its metabolite Galaxolidone and Tonalide were the most abundant compounds. From these evidences, it is clear that additional wastewaters treatments are needed to reduce discharges of synthetic compounds in aquatic systems in order to prevent aquatic ecosystems degradation from prolonged and continuous exposure to these substances.

3.10.P-Th150 The Contribution of Microflow Liquid Chromatography to the Analysis of Micropollutants and their Transformation Products in Sediments

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Analytical chemistry for environmental monitoring constitutes a paradox. Indeed, it is involved in environmental protection through the monitoring of substances in a variety of matrices, such as wastewaters or sediments, while impacting the environment and human health due to toxic reagents, solvents, and energy-intensive instruments like liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). This technique is widely used in environmental analysis due to its ability to separate, identify, and quantify a wide range of micropollutants in complex matrices, even at very low concentrations. This paradox gave birth in 2013 to a new concept, called Green Analytical Chemistry (GAC) which is defined by 12 principles such as minimizing energy consumption, reducing the quantity of solvents and reagents used or miniaturizing methods.

In this context, our goal is to study the contribution of micro-flow liquid chromatography coupled with tandem mass spectrometry ($\mu\text{LC-MS/MS}$) for the analysis of ultra-traces of micropollutants (pharmaceuticals and pesticides) and their transformation products in river sediments. On the one hand, the comparison of μLC and LC concerning analytical performances will be studied. Sensitivities and matrix effects obtained with two similar methods (geometric transfer) will be compared for this purpose. On the other hand, its contribution regarding the reduction of environmental impact will be discussed and quantified with a Life Cycle Assessment (LCA).

This work represents a pioneering step towards the adoption of GAC principles by integrating miniaturized fluidic systems into analytical instrumentation. It aims to show the feasibility and relevance of using $\mu\text{LC-MS/MS}$ in environmental analyses, which is marginal, to reduce the environmental impact of methods while maintaining satisfactory analytical performances. Nevertheless, initial environmental assessments indicate that solely reducing instrumental solvent usage is insufficient to significantly lower the overall environmental impact of analysis. Prioritizing sample preparation is crucial.

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3.10.B Analysis, Assessment and Management of Contaminants of Emerging Concern and Their Transformation Products

3.10.B.T-01 Fate and Occurrence of Quaternary Ammonium Compounds in Aquatic Environments: From Photochemical Transformation of Ionic Liquid Cations to Suspect Screening in Lake Sediments

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Quaternary ammonium compounds (QACs) are a diverse group of organic chemicals with molecular masses ranging from 100 to 600 g/mol and alkyl sidechains of 2 to 22 C atoms. Long-chain QACs are high production volume chemicals that are mainly used as surfactants and biocides in cleaning and personal-care products. Short-chain QACs are used as cations in ionic liquids, an emerging group of nonvolatile solvents with various potential applications in the chemical industry. The fate and occurrence of QACs in aquatic environments is challenging to study because, especially long-chain QACs, are difficult to quantify reliably with standard mass spectrometry methods. Furthermore, the fate of ionic liquid cations, which potentially deviates from long-chain QACs, has received little attention. To improve the assessment of both long- and short-chain QACs, we have (1) developed a high-resolution mass spectrometry method for target and suspect screening of a wide range of QACs, (2) screened wastewater effluent and lake sediment samples for the presence of long- and short-chain QACs, and (3) quantified the photochemical transformation rates of ionic liquid cations in sunlit surface waters. 13 long-chain QACs could be semi-quantitatively determined in both wastewater effluent and surface sediment samples with average total concentrations of 3 µg/L and 3 µg/g, respectively. Short-chain QACs were only detected in wastewater effluent samples with three compounds amounting to an average total concentration of 0.3 µg/L. Comparison of different wastewater treatment trains suggest that the short-chain QACs are less biodegradable than the long-chain QACs. In addition, our laboratory experiments with ionic liquid cations indicate half-lives of 1-4 months for photochemical transformation in sunlit surface waters. Overall, the transformation of short-chain QACs is expected to be even slower than the transformation of long-chain QACs in aquatic environments. Combined with the fact that short-chain QACs were not found in surface sediment samples suggest that these small compounds are also not removed efficiently by sedimentation resulting in a potential for their accumulation in surface and groundwater.

3.10.B.T-02 Efficiency of Sustainable Urban Drainage System in Emerging Pollutants Abatement. Which is the Fate of Mobile Compounds and Transformation Products in these Systems?

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Sustainable urban drainage systems (SUDS) are constructions designed to mitigate the adverse effects of urbanization. The main objective of these systems is to replicate the natural drainage system prior to the paving of cities, but it has more benefits such as promoting aquifer recharge, mitigation of urban heat islands, climate resilience, etc. Among SUDS, bioretention cells are a low-impact technology that mimics the functions of an unurbanized watershed. They are not specifically designed to reduce organic contaminants at trace levels, but have proven to be efficient in removing hydrophobic contaminants such as polycyclic aromatic hydrocarbons, although their efficiency in removing polar organic compounds (highly soluble in water) is expected to be quite low. In addition, contaminants can undergo several degradation processes such as photolysis, oxidation, reduction or biotransformation, and knowledge about the appearance of transformation products in urban stormwater and after passing through SUDS is scarce. In this work, liquid chromatography coupled with high-resolution mass spectrometry was applied to stormwater runoff samples, samples taken after the SUDS, and groundwater samples to identify mobile compounds passing through the SUDS and reaching the aquifer, which contaminants are retained in the SUDS, and transformation products formed due to interactions of the contaminants with the SUDS. For this purpose, stormwater runoff samples were pooled and compared with samples taken after passing through the SUDS. It was found that the SUDS were able to retain polymers such as polyethylene glycol (PEG) or polypropylene glycol (PPG), since PEGs and PPGs of different lengths were found in the runoff

and not after the SUDS. Several compounds such as caffeine, scopoletin, N,N'-Diphenylguanidine, imidacloprid, tris(2-butoxyethyl)phosphate showed the same behavior. Other compounds such as N-butylbenzenesulfonamide and benzoguanamine were significantly reduced by SUDS but were detected in the SUDS samples. On the other hand, compounds such as citroflex A-4, tributyl phosphate, guanylfurea and lidocaine N-oxide showed the same intensity in both sample groups. Also interesting was the finding of fenuron, a herbicide.

3.10.B.T-03 Occurrence of Organic Micropollutants and Transformation Products in Managed Groundwater Aquifer Recharge: from Surface Water Infiltration to Produced Drinking Water *Alessia Ore¹, Merijn Schriks², Annemarie van Wezel³ and Nora B. Sutton¹, (1)Wageningen University, Netherlands, (2)Vitens N.V. Water Expertise Centrum, Netherlands, (3)University of Amsterdam, Netherlands*

Freshwater scarcity is of growing concern in industrialized countries, including the Netherlands, where groundwater is the primary source of drinking water. Recent droughts and intensive drainage have lowered Dutch groundwater tables, prompting drinking water companies and water authorities to explore reuse solutions to replenish aquifers, aiming at alleviating the pressure on natural water sources while maintaining good drinking water quantity and quality.

Managed aquifer recharge (MAR) is widely applied but concerns remain about its effect on natural groundwater quality due to the presence of organic micropollutants (OMPs) and transformation products (TPs) in the infiltrated water, as well as their fate in the environment. This study investigates the impact of MAR with surface water on a Dutch drinking water production area.

Our study assesses water quality changes along the soil passage from infiltration ponds to drinking water production by analyzing water samples of the infiltration pond, surface water, monitoring wells, and extraction wells. Using both target LC-MS and non-target UHPLC-HR-TIMS-MS analysis methods we ensure a comprehensive water quality assessment of known and unknown OMPs and TPs. Additionally, information on the environmental conditions and samples for microbial community analyses were collected.

Preliminary target analysis results indicate significant water quality improvements along the infiltration path from the pond to the closest groundwater extraction well, with removal efficiency of up to 78% of the 25 OMPs detected in the infiltrated water. A few OMPs, including pharmaceuticals (e.g., gabapentin) and pesticide TPs (e.g., dimethenamide-ESA/OA and metolachlor-ESA/OA), remain in extracted water at concentrations around or above 0.1 µg/L. Further treatment at the drinking water production plant further improved water quality. Nonetheless, some OMPs (e.g., TFA, dimethenamide- and metolachlor-ESA) remained in the finished drinking water. The NTA data treatment and interpretation (currently work in progress) will provide additional elucidation on (unknown) TPs and water quality changes along the infiltration path, also in correlation with environmental parameters.

Our research will provide novel findings for a comprehensive exposure assessment of OMPs' (including TPs) presence in water reuse scenarios. These insights can support improved decision-making, regulations, and environmental risk assessment for safer water reuse.

3.10.B.T-04 Pesticides as a Source of Trifluoroacetate (TFA) to the Environment

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Compounds containing at least one carbon-bound trifluoromethyl moiety (C-CF₃) are potential precursors of the very persistent and very mobile substance trifluoroacetate (TFA). Despite their broad and increasing application, there are no comprehensive studies that have focused on the role of pesticide as a source of TFA to the (aquatic) environment. To address this research gap, the TFA formation potential from agricultural pesticide use was calculated for Europe and the contiguous USA using sales volume estimates of C-CF₃-containing pesticides. In addition, spatially resolved monitoring data were used to correlate groundwater TFA concentrations with land use categories and nitrate concentrations in Germany to elucidate the influence of pesticide application on groundwater TFA concentrations. Finally, historical (1940s-2000s) and recent wine samples from Europe were analyzed to determine long-term temporal trends of TFA in wine. Pesticides with TFA formation potential were found to vary in type and use profile

between regions, with the estimated maximum TFA emissions ranging from 0 to 83 kg/(km² × year). Based on a molar TFA yield of 100%, a total of 3,200 t and 5,000 t of TFA could be emitted from agricultural pesticide use in Europe and the USA, respectively. Compound-specific TFA formation rates and field studies are needed to characterize the formation of TFA from precursors under environmental conditions and to rank and prioritize pesticides of concern for potential (regulatory) action. Our evaluations revealed a significantly ($p < 0.001$) higher average TFA concentration in the samples of groundwater bodies located in agricultural areas (mean = 1.59 µg/L; median = 1.1 µg/L; $n = 411$; proportion of agricultural land use >50%) than in areas with lower degrees of agricultural activity. Moreover, the average TFA concentration in the groundwater samples containing nitrate concentrations >25 mg/L (mean = 1.84 µg/L; median = 1.30 µg/L; $n = 556$) was significantly ($p < 0.0001$) higher than in samples containing ≤25 mg/L of nitrate (mean = 0.92 µg/L; median = 0.53 µg/L; $n = 1021$). This strongly suggests that agricultural land use is associated with elevated TFA concentrations in the aquatic system. The preliminary analysis of wine samples showed a strong positive temporal trend in TFA concentration. This is another indication of the substantial increase of anthropogenic emissions of various TFA precursors, including C-CF₃ containing pesticides, over the last decades.

3.10.P-Th145 PMTfocus: Tracing Persistent, Mobile, and Toxic Substances from Water Resources to Human Exposure – An Emerging Environmental and Public Health Challenge

Julietta Sturla Lompre¹, Geoffroy Duporte¹, Clementine Gavalon¹, Teo Ferreux¹, Eleonore Resongles¹, Chirstelle Batiot-Guilhe¹, Nassim Ait Mouheb², Fethi Lachaal³, Helene Fenet¹, Frederique Courant¹ and Elena Gomez¹, (1)HydroSciences Montpellier, IRD, CNRS, University of Montpellier, France, (2)UMR G-EAU - Gestion de l'Eau, Acteurs, Usages; INRAE - Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, France, (3)Georesources Laboratory, Water Research and Technology Centre of Borj Cedria, Tunisia

Persistent, mobile, and toxic (PMT) substances in water systems represent a significant environmental and public health challenge. Their chemical properties (resistance to natural degradation and conventional treatment methods) enable their accumulation and dispersion in groundwater, surface water, and drinking water. Recognizing their threat, the European Union classified PMT and vPvM substances as hazard classes under the Classification, Labelling and Packaging of Chemicals Regulation in 2021, in alignment with the EU Drinking Water and Groundwater Directives. The interdisciplinary PMTfocus project investigates PMTs across diverse environments, assessing their occurrence, behavior, and pathways to human exposure.

Samples were collected from four strategic locations: Lez Spring, France (low urban pressure reference); Gherdaia, Algeria (wastewater-impacted aquifers); Tunisia (artificial recharge with treated wastewater); and Oruro, Bolivia (urban and mining pollution). Analytical methods combined targeted LC-HRMS and non-targeted screening using Solid Phase Extraction (HLB and WAX cartridges). Targeted analyses quantified 51 PM/PMT compounds, including carbamazepine, detected in Tunisia (up to 409.1 ng/L), Bolivia (70.7 ng/L), and Algeria (119.5 ng/L), but below detection limits in Lez Spring. Non-targeted screening identified hundreds of compounds in Tunisian groundwater, 35 of which were confirmed (with analytical standards) as PM/PMTs, including carbamazepine metabolites.

PM/PMTs, including pharmaceuticals, pesticides, industrial chemicals, and personal care products, were detected at all sites. Anthropogenic pressures, particularly urbanization and inadequate wastewater management in Bolivia and Algeria, rise water contamination. The dual approach of targeted and non-targeted analyses ensures comprehensive monitoring of known and emerging contaminants, providing insights into their persistence, behavior, and risks in aquatic systems.

Correlating water analysis with urinary biomarkers will evaluate human exposure, linking environmental contamination to health risks. Results underscore the urgent need for regulatory improvements to mitigate PMT impacts, particularly in regions practicing water reuse or relying on untreated sources. The PMTfocus project contributes to advancing sustainable water management practices, protecting ecosystems and public health globally.

3.10.P-Th146 From Wastewater to Rivers and Soils – Particle-Associated Emissions of Quaternary Ammonium Compounds

Sophie Lennartz¹, Jan Koschorreck², Collin Weber³, Bernd Gockener⁴, Karlheinz Weinfurter⁴, Andrea Frohbose-Korner², Jan Siemens⁵, Sanjana Balachandran⁶, Stefanie P. Glaeser⁶ and Ines Mulder⁷, (1)Institute of Soil Science and Soil Conservation, Justus-Liebig University Giessen, Germany, (2)German Environment Agency (UBA), Germany, (3)Institute of Applied Geoscience, Technical University of Darmstadt, Germany, (4)Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany, (5)Institute of Soil Science and Soil Conservation, Justus-Liebig-University Giessen, Germany, (6)Institute of Applied Microbiology, Justus-Liebig-University Giessen, Germany, (7)Institute of

Geography, Ruhr-University Bochum, Germany

Quaternary ammonium compounds (QACs) are antimicrobials and cationic surfactants discharged into the environment via municipal, industrial or livestock wastewater. Recently, concerns about their aquatic ecotoxicity and potential contribution to selection and spread antimicrobial resistance have increased, especially since the SARS-CoV-2 pandemic. Although wastewater treatment plants (WWTPs) largely remove QACs from the aqueous wastewater phase, we hypothesized that due to their strong surface affinity, QACs will partition primarily to suspended solids and enter the downstream environment associated with discharged particles. In wastewater-impacted rivers, QACs are expected to be transported in the suspended particulate matter (SPM) a vector for their sedimentation and accumulation in floodplain soils. Moreover, we hypothesized that the concentrations and ecotoxicological risks of QACs in SPM increased during the SARS-CoV-2 pandemic due to the drastic surge in disinfectant use.

We measured the distribution of QACs between the solid and liquid phases of activated sludge and wastewater of three German WWTPs. Concentrations of QACs were determined in SPM collected by the German Environmental Specimen Bank from three German rivers with varying wastewater shares between 2006-2021, and across different depths of floodplain soils. Using targeted multi-residue HPLC-MS/MS analysis, 30 different QACs were quantified including alkyltrimethyl, benzylalkyl and dialkyldimethyl ammonium compounds of varying chain lengths. Ecotoxicological risks in SPM and soil were estimated based on predicted no-effect concentrations and minimal inhibitory concentrations. Increasing with their hydrophobicity, QACs showed preferential particle-association in WWTPs and were found in all analyzed SPM samples with concentrations up to mg kg⁻¹. High spatiotemporal variability suggests the wastewater share of rivers and seasonal dynamics as important drivers of QAC concentrations in SPM, exceeding the presumed imprint of the pandemic. QAC homologue distributions in the upper layers of floodplain soils closely resembled those in SPM, confirming the importance of particle-associated transport in determining the environmental distribution of QACs. For both SPM and soils, ecotoxicological risks cannot be excluded. This highlights QACs as contaminants of emerging concerns at the aquatic-terrestrial interface.

Disclaimer/Disclosure: This study was funded by the German Research Foundation (DFG) project number 458460392.

3.10.P-Th173 Water Treatment Processes: Identification and Quantitation of Transformation Products Formed in Test Systems

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A recent European Food Safety Authority (EFSA) guidance document describes experimental methods for the identification of transformation products of active substances and their metabolites formed during water treatment processes. These processes include chlorination, chloramination, oxidation, ozonolysis, and ultraviolet light (UV) treatment. Here, we present results of experimental implementation of these processes carried out with a series of test substances.

Experiments were carried out in a standard water with addition of organic carbon obtained from the Suwannee River as defined in the guidance document. Chlorination with sodium hypochlorite was carried out at three different pH levels (6.5, 7.5, and 8.5). Monochloramine was prepared from sodium hypochlorite and ammonium chloride to carry out the chloramination experiments. For the oxidation process, an aqueous chlorine dioxide solution was obtained by addition of sulfuric acid to a solution of sodium chlorite.

In all of these experiments, the concentration of reagent in the test system was determined using the colorimetric N,N-diethyl-p-phenylenediamine (DPD) method to ensure that the requirements of the guidance document were met.

Ozonolysis was carried out by treating the test substances with a freshly prepared solution of ozone in water, while the UV process was performed in an irradiation chamber fitted with a medium pressure UV lamp allowing control of the UV dose to the test solution.

Liquid chromatography coupled with mass spectrometry (LC/MS) was used to analyse the test substances and transformation products formed in these processes. Identification of the transformation products was carried out using high resolution-accurate mass spectrometry (HRAM) with data analysis performed using a non-target screening workflow, while quantitative analysis was performed by HRAM or multiple reaction monitoring (MRM) on a triple quadrupole mass spectrometer.

The results of these experiments are presented, with a discussion of challenges and suggested approaches to fulfilling the requirements of the guidance document.

3.10.P Analysis, Assessment and Management of Contaminants of Emerging Concern and Their Transformation Products

3.10.P-Th143 The Effect of Lignin Derivative Addition as Co-Substrate on DOC Dynamics and OMP Biodegradation in Drinking Water Aquifers

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This research explores the effects of adding a lignin derivative, 4-HBA, on DOC dynamics and OMP biodegradation in drinking water aquifers. Conducted at five different aquifer locations with varied water residence times (WRTs), the study involved two sets of batch experiments per location one with natural DOC and another with added 4-HBA over a 90-day aerobic incubation period. Although initial DOC quality showed no spatial differences, temporal EEM analyses indicated a decline in DOC fluorescence, suggesting a long-term biodegradation of humic substances.

While the addition of 4-HBA initially boosted microbial activity, marked by significant changes in the fluorescence of microbial byproducts, this enhancement did not lead to improved OMP biodegradation. Instead, the presence of 4-HBA appeared to inhibit biodegradation relative to batches with only natural DOC. This inhibitory effect could be attributed to several factors, including potential carbon catabolite repression, alterations in microbial community composition, and diauxic growth patterns that might extend the time needed for microbes to adapt to new substrates. These findings indicate that although 4-HBA serves as an easily consumable carbon source, its incorporation may complicate the microbial processing of OMPs.

In summary, the addition of 4-HBA, while stimulating initial microbial activity, has an inhibitory impact on OMP biodegradation. This underscores the complexities of introducing external carbon sources into aquifer systems and emphasizes the importance of a nuanced understanding of microbial and chemical dynamics for effective bioremediation strategies.

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3.10.P-Th144 Investigating the Presence of Emerging Contaminants in GB Honey

John Nightingale¹, Laura Carter², Narmin Garazade², Ben Woodcock³ and Jennifer Shelton³, (1)University of Leeds, Leeds, United Kingdom, (2)The University of Leeds, United Kingdom, (3)CEH, United Kingdom

The application of animal manures and biosolids is a well-established practice for waste disposal and soil fertility improvement. However, these practices can inadvertently release emerging contaminants into terrestrial and aquatic environments. Some of these contaminants, including human and veterinary pharmaceuticals, can persist in soils and accumulate in crops, although plant uptake is influenced by environmental parameters and the physiochemical properties of the chemical. Research on the translocation of these contaminants to pollen and nectar, and ultimately to beehives, is limited. It remains unclear whether emerging contaminants, such as human and veterinary pharmaceuticals, are accumulating in hives and potentially exerting toxicological effects as a result of these practices, this gap in our knowledge is surprising, especially given the increasing use of biosolids, which are well-known to be contaminated with a range of pharmaceuticals. Therefore a pilot study was conducted on honey samples collected from sites with 70% arable land use within a 2 km radius (n = 30 sites). A modified QuEChERS extraction technique coupled with Non-target screening (HRMS) was employed and was capable of identifying four main contaminant groups, these were human pharmaceuticals (50%), industrial contaminants (33.3%), agri-chemicals (16.7%), and veterinary pharmaceuticals (0.8%) in an array of the samples. The presence of the fungicide azoxystrobin was expected, but, to the best of our knowledge, this is the first report of pharmaceuticals such as ibuprofen, flurandrenolide, alprenolol, propranolol, and aspirin in honey samples. Semi-quantitative analysis revealed concentrations of human pharmaceuticals ranging from 0.015 to 0.91 µg/g (dw). The toxicological effects of these concentrations on human and forager health remain unclear. Therefore this research demonstrates an appropriate means to target chemicals of concern in agri-ecosystems but also to provide assessments of risk to bee health. These findings are substantial and reiterate the need for a better understanding of the risks and factors contributing to declining bee populations. They warrant urgent attention and further investigation.

3.10.P-Th145 PMTfocus: Tracing Persistent, Mobile, and Toxic Substances from Water Resources to Human Exposure – An Emerging Environmental and Public Health Challenge

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3.10.P-Th146 From Wastewater to Rivers and Soils – Particle-Associated Emissions of Quaternary Ammonium Compounds

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(1)Institute of Soil Science and Soil Conservation, Justus-Liebig University Giessen, Germany, (2)German Environment Agency (UBA), Germany, (3)Institute of Applied Geoscience, Technical University of Darmstadt, Germany, (4)Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany, (5)Institute of Soil Science and Soil Conservation, Justus-Liebig-University Giessen, Germany, (6)Institute of Applied Microbiology, Justus-Liebig-University Giessen, Germany, (7)Institute of Geography, Ruhr-University Bochum, Germany

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3.10.P-Th147 Effect-Based Strategy for Identification and Assessment of Potential Health Effects of Hazardous Organic Chemicals in Vehicles

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The car cabin is an indoor environment of interest due to the high material-to-space ratio and the high temperatures that can be reached, especially during a sunny summer s day. Plastic materials of various sources and composition is the predominant material inside vehicles, which is concerning due to the lack of information about what potential hazardous chemicals may contain. In this project, active air sampling was performed in a stand-still car at 25°C vs 65°C in a climate chamber. In addition, solvent and heat extraction of plastic materials, used in vehicles, was performed separately to investigate the sources of the chemicals and biological effects seen in the air samples.

The air samples were chemically and biologically characterised using GC/LRMS and different in vitro Chemical Activated LUCiferase gene Expression (CALUX®) bioreporter assays. The chemical target method included different groups of polycyclic aromatic hydrocarbons e.g., native PAHs, alkyl-PAHs and oxy-PAHs. To further identify chemicals in the air and materials, suspect screening using high-resolution GC/HRMS and LC/HRMS will be performed on the samples. Pesticides, flame retardants, plasticisers and other plastic associated chemicals will be a part of the suspect list. The in vitro bioassays used for analysing air and material samples are ER-, anti-AR, DR- and Nrf2-CALUX®. Additionally, the plastic materials will be analysed for the presence of PFAS compounds using a LC/MS method targeting PFAS compounds, and CIC measuring extractable organic fluorine.

Preliminary results from the climate chamber shows that the highest activities can be seen in the bioassays when the temperature is increased to 65°C, and that a decrease in activity can be seen over the following days when heating up the same car repeatedly. This suggests that the release of semi-volatile compounds from materials will decrease over time when the vehicle is being heated. Only a few extractions of plastic materials have been performed at this time. Results from these show that the materials contain compounds which are active in both DR- and ER-CALUX.

The results from this study will increase the knowledge about the identity, sources and the combination effects of chemicals present in the car cabin environment as well as appropriate methods to screen for the chemicals.

3.10.P-Th148 Seasonal Variation of Contaminants of Emerging Concern in Swedish Landfill Leachate and its Recipient: A Case Study

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Landfill leachate is an important point source of contaminants of emerging concerns (CECs) in fresh water systems. There are over 350 active landfills in Sweden, producing 8 ~12 million m3 of leachate each year. This study conducted non-target screening (NTS) and suspect screening (SS) using ultra high-performance liquid chromatography coupled with high resolution mass spectrometry (UHPLC-HRMS) to identify the CECs in Swedish landfill leachate. The aim of this research is to map the occurrence of CECs in Swedish landfill leachate and provide insights into their seasonal changes.

In our study, 24-hour composite samples of untreated, treated leachate and recipient upstream, and downstream (named UL, TL, RU, and RD) were collected from a municipal landfill in Sweden in September 2023, January, March, and June 2024 by time-integrated autosamplers. Extraction was performed on multi-layer solid phase extraction (SPE) cartridges in order to capture a wide spectrum of CECs. Preliminarily, 74 CECs were identified in all the landfill leachate samples, including 35 industrial chemicals, 21 pharmaceuticals, 10 PFASs, and 9 pesticides. Winter had the highest number of detected CECs and the highest detection frequency among all seasons. 49 CECs were detected in UL, TL, and RD across all seasons, and 16 of them were also detected in RU in either or all seasons. Some industrial chemicals (e.g., tripropyl phosphate and dibutyl phthalate) showed lower detection frequencies in warmer seasons (summer and/or autumn), while some pharmaceuticals, such as bicalutamide, losartan, and lamotrigine, had lower detection frequency in spring and summer. Possible reasons for the seasonal

change in the occurrence of CECs can be: (1) increased removal efficiency at higher temperature; (2) the amount of produced leachate and the concentration of CECs can be influenced by precipitation; (3) the reception of external waste may vary depending on time. These findings highlight the need for seasonal monitoring of CECs and adjustments of leachate treatment strategies to manage the diverse presence of CECs in landfill leachate throughout the year.

3.10.P-Th149 Synthetic Musk: From Wastewaters to Surface Water Ecosystems

Stefano Tasselli and Licia Guzzella, CNR-IRSA, Italy

Synthetic musks are compounds incorporated in detergents, perfumes, and house-cleaning products. Within them there are Galaxolide, its metabolite Galaxolidone, Tonalide, Celestolide and Phantolide. They are released every day in large amounts into sewage systems and discharged in aquatic ecosystems through wastewater treatment plant (WWTP) effluents. The prolonged exposure of aquatic environments to the continuous discharge of PMFs is raising many concerns about their possible chronic toxicity and future impacts. Data regarding PMFs in Italy are very scarce even if this is the EU-Member State with the highest consumption of these chemicals. In this study, we sampled wastewater in four different stations of a conventional WWTP with activated sludges to understand fate and behaviour of PMFs during treatments. PMFs were measured in all stations up to the $\mu\text{g/L}$ order of magnitude. Biotransformation and adsorption were the two processes driving the fate of these contaminants during treatments. High and stable musk concentrations were measured in activated sludges. Only a slight reduction of the main PMFs, Galaxolide (-20%) and Tonalide (-30%), was evidenced during treatments, together with the increase of the Galaxolide metabolite (+70%). This leads to discharges of these compounds in receiving water bodies which can be degraded by WWTP effluents. PMF presence was then assessed in an anthropized surface water ecosystem, Lake Maggiore, considering both water and sediments of the main tributaries to investigate concentrations entering the lake but also two freshwater bivalves to assess possible bioaccumulation. In waters, characterized also in terms of pH, conductivity, alkalinity, major ions and nutrients, PMFs were detected up to hundreds of ng/L especially in small rivers in which the highest values of N and P were measured. Sediment analyses followed the same degree of pollution with concentration peaks detected during summer seasons. Molluscs analysis reflected the degree of anthropic pressure present on the lake shores, highlighting two different bioaccumulation patterns in the two considered species. In all matrices, Galaxolide, its metabolite Galaxolidone and Tonalide were the most abundant compounds. From these evidences, it is clear that additional wastewaters treatments are needed to reduce discharges of synthetic compounds in aquatic systems in order to prevent aquatic ecosystems degradation from prolonged and continuous exposure to these substances.

3.10.P-Th150 The Contribution of Microflow Liquid Chromatography to the Analysis of Micropollutants and their Transformation Products in Sediments

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Analytical chemistry for environmental monitoring constitutes a paradox. Indeed, it is involved in environmental protection through the monitoring of substances in a variety of matrices, such as wastewaters or sediments, while impacting the environment and human health due to toxic reagents, solvents, and energy-intensive instruments like liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). This technique is widely used in environmental analysis due to its ability to separate, identify, and quantify a wide range of micropollutants in complex matrices, even at very low concentrations. This paradox gave birth in 2013 to a new concept, called Green Analytical Chemistry (GAC) which is defined by 12 principles such as minimizing energy consumption, reducing the quantity of solvents and reagents used or miniaturizing methods.

In this context, our goal is to study the contribution of micro-flow liquid chromatography coupled with tandem mass spectrometry ($\mu\text{LC-MS/MS}$) for the analysis of ultra-traces of micropollutants (pharmaceuticals and pesticides) and their transformation products in river sediments. On the one hand, the comparison of μLC and LC concerning analytical performances will be studied. Sensitivities and matrix effects obtained with two similar methods (geometric transfer) will be compared for this purpose. On the other hand, its contribution regarding the reduction of environmental impact will be discussed and quantified with a Life Cycle Assessment (LCA).

This work represents a pioneering step towards the adoption of GAC principles by integrating miniaturized fluidic systems into analytical instrumentation. It aims to show the feasibility and relevance of using $\mu\text{LC-MS/MS}$ in environmental analyses, which is marginal, to reduce the environmental impact of methods while maintaining satisfactory analytical performances. Nevertheless, initial environmental assessments indicate that solely reducing instrumental solvent usage is insufficient to significantly lower the overall environmental impact of analysis. Prioritizing sample preparation is crucial.

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3.10.P-Th151 In Silico Tentative Identification of Pharmaceutical Biotransformation Products in Receiving Water

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Pharmaceuticals and their biotransformation products (BPs) are not monitored routinely in river water. A European Commission report in 2020 details a shortlist of several anti-microbial pharmaceuticals in the next EU Water Framework Directive (WFD) watch list to be closely monitored in surface waters.

Antimicrobials such as sulfamethoxazole and clotrimazole are pharmaceuticals that are of particular concern, increasingly being detected in surface water and soils across Europe and are toxic and mobile in the aquatic environment.

This work aims to fill a research gap in the lack of predictive understanding of pharmaceutical biotransformation (BT) and degradation; high resolution analytical methods have been developed for in silico ID of pharmaceuticals and their BPs but do not provide information about the mechanisms of BT. It will determine the BT rate of prioritised compounds (including those in the EU WFD watch list) and investigate their BT pathways in river water. The microbial and chemical data derived from these studies will be used to create a model that can help predict the BT of pharmaceuticals in river water.

Suspect screening in HRMS analysis has been used to detect and identify APIs and their BPs in wastewater impacted rivers and the use of online predictive tools such as BioTransformer 3.0 (BT 3.0) and EAWAG BBD/PPS have been used to predict BPs that could be formed in environmental microbial transformation (EMT). Structures of these predicted BPs are compared with spectral libraries and machine learning based retention time (tR) prediction models to shortlist suspect pharmaceuticals. 36 out of 900 BPs predicted using BT 3.0 and EAWAG-BBD/PPS were found in HRMS data at the most wastewater impacted site, as well as 22 BPs in unspiked riverwater and 66 BPs in spiked riverwater at that same site. Trimethoprim, sulfamethoxazole, citalopram, propranolol and venlafaxine (observed in WFD watchlist) have BPs that have lower PNEC values than their parent compounds in freshwater, which may allude to higher environmental risk in water.

Future work includes the application of the tR model to BPs of these compounds in HRMS data, as well as assessing identified BPs in terms of environmental risk e.g., PNEC. Biological analytical methods, e.g., qPCR and biological predictive databases e.g., PathPred, BRENDA will also be conducted to observe resident enzymes and/or microbial communities that may be involved in the observed BTs of these specific compounds.

3.10.P-Th152 Temporal Characterization of Progestins Accumulation in Three Different Estuarine Systems Along the Portuguese Coast

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Synthetic progestins are micropollutants of special concern, due to their growing use in human and veterinary therapies and their risks to aquatic life. Currently, there is a lack of environmental information on these compounds, worldwide. The main objective of this work was to characterize the levels of the most consumed progestins in Portugal. For that, Ria de Aveiro, Tagus estuary and Ria Formosa were sampled in a temporal perspective to evaluate levels of drospirenone (DRO), desogestrel (DSG), gestodene (GST) and levonorgestrel (LNG) in surface water samples and macrobenthic organisms (bivalve, polychaete, and crustacean). Regarding surface waters, drospirenone and desogestrel were the most abundant progestins. In the North of Portugal, DSG was the most abundant (Aveiro: 193.9 ng L⁻¹, summer), while DRO was more representative in the South (Tagus: 178.9 ng L⁻¹; Formosa: 125.7 ng L⁻¹), also in summer. These spatial differences can be associated with the hydrodynamics of each estuarine system as well as the distinct population and tourist levels associated with each site.

Concerning the biological matrices, progestins were only quantifiable for the crustacean. Values were generally low, peaking with drospirenone values in Ria de Aveiro (1.33 ± 0.26 ng/g ww) and Tagus estuary (1.42 ± 0.55 ng/g ww), while Ria Formosa exhibited the lowest progestin concentrations (< 1 ng/g ww).

In conclusion, it is essential to maintain ongoing monitoring of aquatic systems, particularly regarding

progesterone levels, not only in surface waters but also in other matrices such as sediments and biota. This will enhance our understanding of the true impact of these compounds and provide critical information to support the development of regulations aimed at controlling hormone levels in the environment.

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3.10.P-Th153 Pharmaceutical Removal in Batch Anaerobic Wastewater Treatment: Ecotoxicological Impacts on Cladocerans as Bioindicators

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Pharmaceuticals can cause toxic effects on various aquatic organisms and, therefore, they must be removed in wastewater treatment plants. Anaerobic bioreactors have shown promise in degrading these compounds, particularly during the acidogenic phase. This study aimed to evaluate the ecotoxicity of synthetic effluents before and after treatment in batch anaerobic bioreactors. The treatment process involved two scenarios: one with and one without the inoculation of eight target compounds (acetaminophen, atenolol, caffeine, carbamazepine, diclofenac, ibuprofen, naproxen, and propranolol). Treatment efficiency was assessed using two species of cladocerans, namely *Ceriodaphnia silvestrii* and *Daphnia similis*. Both species were cultured following Brazilian standards (ABNT NBR 13373/2022 and 12713/2022). Acute ecotoxicity assays were performed with effluent samples at 100% and 50% concentrations (10 mL for *C. silvestrii* and 15 mL for *D. similis*), using four replicates with five neonate organisms per test. The assays lasted 48 h at 25°C for *C. silvestrii* and 20°C for *D. similis*, under artificial light (100 lx) and controlled photoperiods (12h: 12 h for *C. silvestrii* and 16h: 8 h for *D. similis*). Validation was achieved when control samples exhibited less than 20% immobility, and data was compared by Analysis of Variance (ANOVA, with 95% confidence level). Despite the reduction in pharmaceutical concentrations in effluents, *C. silvestrii* had significant immobility when exposed to 50% and 100% samples, likely due to the high salinity of synthetic wastewater, reflecting this species' sensitivity to such conditions. For *D. similis*, organisms remained mostly mobile at 50%, with no significant differences observed between effluents with and without pharmaceuticals ($p > 0.05$). However, at 100%, samples with pharmaceuticals were more toxic, with a mobility of $75 \pm 5\%$, compared to $47 \pm 3\%$ in samples without these compounds. Despite high effect concentrations reported for individual compounds (generally in the order of mg/L), factors such as compound mixtures and elevated salinity may contribute to the overall toxicity of treated samples. Even after the removal of pharmaceuticals, their transformation products (TPs) may still pose a toxic threat. The results reinforce the necessity of ecotoxicity assessments for wastewater effluents, particularly considering the complexity of wastewater monitoring and the potential risks posed by pharmaceutical mixtures and their by-products.

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3.10.P-Th154 Quantification and Environmental Fate of Pharmaceuticals and Personal Care Products Across the Soil and Water Systems of Delhi: Insights from Batch Experiments

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Emerging contaminants such as pharmaceuticals and personal care products (PPCPs) pose significant ecological and health risks due to their persistence, bioactivity, and potential transformation into toxic metabolites. The National Capital Region (NCR) of Delhi, India intersected by the heavily polluted Yamuna River represents a critical case study for understanding the environmental behavior of PPCPs, as the region is impacted by untreated wastewater and industrial effluents.

This study focuses on the analysis and fate of 32 PPCPs spanning diverse classes, including antibiotics, hormones and endocrine disruptors, analgesics and non-steroidal anti-inflammatory drugs (NSAIDs), anti-allergens, anti-epileptics, stimulants, beta-blockers, lipid regulators, antibacterials, and artificial sweeteners. A robust and validated liquid chromatography-mass spectrometry (LC-MS) method has been developed for precise quantification of these compounds in soil, groundwater, and surface water matrices. In addition, transformation products and metabolites of the targeted compounds will be screened using Quadrupole Time-of-Flight (Q-TOF) mass spectrometry.

To understand the environmental behavior of PPCPs, systematic batch experiments will be conducted to investigate sorption and desorption kinetics, as well as isotherms, in soils from the study region. These experiments provide critical insights into the mobility and bioavailability of PPCPs, highlighting their potential for groundwater contamination. Furthermore, aerobic and anaerobic soil incubation studies will be performed to evaluate the degradation behavior of selected PPCPs under different environmental conditions. For compounds exhibiting significant degradation, Q-TOF will be used to identify transformation products generated under these environmental conditions.

The quantification of PPCPs across the NCR region in soil, groundwater, and surface water will provide critical insights into their spatial distribution, persistence, and transformation pathways. This research will advance our understanding of PPCP behavior in aquatic and terrestrial systems and offer actionable insights for mitigating environmental and health risks while contributing to improved pollution management strategies.

3.10.P-Th155 Impact of Variable Parameters on the Formation of Possible Transformation Products during Drinking Water Treatment Simulation

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Drinking water is prepared from raw water abstracted from environmental sources. Various drinking water treatment techniques need to be applied to achieve a desired level of disinfection. The raw water often contains plant protection products and biocides from various sources such as agriculture and urban infrastructure. Consequently, those chemicals are exposed to disinfection treatments potentially leading to formation of transformation products (TPs), which might not be monitored and could possess toxic properties.

The aim of the Guidance document on the impact of water treatment processes on residues of active substances or their metabolites in water abstracted for the production of drinking water, published by EFSA (2023), is to provide a comprehensive account of experiments that simulate potential drinking water treatments and assess the resulting TPs. The document specifies minimum or maximum values for parameters, e.g., the ratio of the treatment reagent to the active substance or reaction times for the various treatments. On the other hand, the same guideline fixes the volume of water to be used for the experiments very strictly.

The objective of this work is to identify the parameters that are critical for the formation of TPs. The following two hypotheses were tested. Firstly, it was assumed that the significant variation in the ratio of the removal agent and reaction time may exert a considerable impact on the formation of TPs. Secondly, it was hypothesized that the variation in sample volumes has no effect on the formation of TPs.

Terbutryn, a biocide, serves as the model substance for this study. It has already been frequently detected in surface and groundwater, and is, additionally, listed as substance to be monitored in accordance with the Water Framework Directive. The aforementioned propositions are to be conducted by varying the ratio of disinfection agent vs. parent compound, sample volume and reaction time as part of a design of experiment (DOE) and followed by non-target analysis utilizing high resolution mass spectrometry. This is intended to determine the number of features representing possible TPs depending on the parameter variation and to compare them statistically with other variations.

Specifically, this work should provide insight and understanding into whether a careful selection of the precise specification of parameters such as the disinfection agent to parent compound ratio, as well as reaction duration, is necessary.

3.10.P-Th156 In Vitro Dermal Absorption of Key Colour Developers in Thermal Paper: A Focus on Bisphenol A, Bisphenol S, and Pergafast 201

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Thermal papers have been used since the 1970s to print tickets, receipts, but also food packaging labels. Bisphenol A (BPA) has for long been the most common colour developer in thermal papers, but its endocrine-disrupting properties have led to restrict its use in the European Union (it should not exceed 0.02% of thermal printing papers weight). In response, manufacturers have begun to replace BPA with

alternatives such as Bisphenol S (BPS) and other substances, including Pergafast 201 (PF201). BPS was demonstrated to be at least equally as active as BPA, and was classified as an endocrine disruptor in 2023. In contrast, toxicity data for PF201 still lacks. The handling of thermal papers represents the primary route of occupational exposure to colour developers, but also concerns consumers, through receipts and food packaging tags manipulation and storage. Despite the increasing use of BPA alternatives, the percutaneous absorption of these substances remains poorly understood.

We carried out an ex vivo study in accordance with OECD (n428, n28) and EFSA guidelines to evaluate the human dermal absorption of BPA, BPS and PF201. Viable split-thickness abdominal skin samples from women aged 23-48 years (BPS: n = 5 donors; BPA and PF201: n = 7 donors) were mounted on static Franz diffusion cells. Tritium-labelled molecules were topically applied (1.38 nmol/cm²) in phosphate-buffered saline. After 24h, radioactivity distribution was measured in all skin compartments, allowing to establish a mass balance. Mean recoveries were 98.0%, 93.5% and 95.3% of the applied dose, for BPA, BPS and PF201, respectively. The absorbed doses were $4.59 \pm 2.18\%$, $0.56 \pm 0.90\%$, and $0.19 \pm 0.10\%$ for BPA, BPS and PF201, respectively. Average PF201 and BPS absorbed doses were significantly lower compared to the average BPA absorbed dose. Furthermore, dermal delivery values were $15.7 \pm 3.68\%$ (BPA), $7.03 \pm 3.14\%$ (BPS) and $5.31 \pm 5.35\%$ (PF201). Our study provides evidence that these three compounds can reside in the deeper layers of the stratum corneum for more than 24 hours, and thus remain potentially bioavailable.

Overall, this study contributes to a better understanding of dermal exposure to BPA alternatives, with this route being suspected to significantly contribute to human exposure for these substances. Our results may help reach a better assessment of associated hazards and risks by public authorities.

3.10.P-Th157 Assessing Biochar's Potential for Long-Term Pharmaceutical Adsorption in Flow-Through Wastewater Systems

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Conventional wastewater treatment facilities have low removal efficiencies for pharmaceuticals, which are present globally in groundwater and surface water, including drinking water reservoir. This study investigates the adsorption capacities of five different biochar materials for the removal of pharmaceuticals from effluent from tertiary treatment of municipal wastewater, using a continuous flow column experiment to evaluate breakthrough points and removal efficiencies.

The experiment involved exposing biochar materials in a constant flow column system to spiked wastewater effluent containing selected pharmaceuticals with varied physicochemical properties to assess adsorption effectiveness. Among the biochar materials tested, forest biomass and sewage sludge biochar materials demonstrated superior adsorption capacities, maintaining over 99% removal efficiency beyond 440 bed volumes, attributed to higher packing densities. In contrast, spruce biochar displayed a quicker breakthrough due to lower density, underscoring the critical role of biochar characteristics in adsorption performance. Additionally, sludge biochar exhibited biofilm formation, suggesting enhanced biodegradation potential for prolonged treatment applications.

These findings indicate that biochar's surface properties, particularly density and charge, are significant for pharmaceutical adsorption, with cationic pharmaceuticals showing improved retention. This study advances the understanding of biochar-based quaternary wastewater treatment, though further research, including pilot-scale studies, is recommended to assess biochar's long-term efficacy and cost-effectiveness in environmental applications.

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3.10.P-Th158 Screening of Traffic Related Organic Micropollutants in Municipal Stormwater Ponds

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Stormwater ponds are designed to collect water from road runoffs and frequently serve as hot spots for traffic related organic micropollutants (TR-OMP). These pollutants, including their transformation products, can be highly toxic and have a large impact on the environment.

In this study 11 stormwater ponds were investigated around the Uppsala municipality (Sweden). These include potentially impacted sites, remote locations as well as recreational parks. Surface water was collected during three different seasons: spring, summer and fall. Samples were extracted by using a

generic Solid Phase Extraction (SPE) method and analysed by means of ultra-performance liquid chromatographic (UPLC) system coupled to a Q Exactive Focus Orbitrap.

Suspect screening of the collected stormwater samples identified a diverse array of TR-OMP commonly linked to road runoff, including tire wear compounds. This analysis highlights the diversity of the contaminant profiles across different stormwater pond locations, suggesting that factors such as urban density, traffic intensity, and local land use significantly influence the pollutant profiles. The variability in TR-OMP composition underscores the complex interplay between stormwater dynamics and environmental exposure pathways, emphasizing the need for site-specific monitoring and tailored mitigation strategies to manage stormwater quality effectively.

3.10.P-Th159 Pesticide Residues with Hazard Classifications Relevant to Non-Target Species Including Humans are Omnipresent in the Environment and Farmer Residences

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Intensive and widespread use of pesticides raises serious environmental and human health concerns. The presence and levels of 209 pesticide residues (active substances and transformation products) in 625 environmental samples (201 soil, 193 crop, 20 outdoor air, 115 indoor dust, 58 surface water, and 38 sediment samples) have been studied. The samples were collected during the 2021 growing season, across 10 study sites, covering the main European crops, and conventional and organic farming systems. We profiled the pesticide residues found in the different matrices using existing hazard classifications towards non-target organisms and humans. Combining monitoring data and hazard information, we developed an indicator for the prioritization of pesticides, which can support policy decisions and sustainable pesticide use transitions. Eighty-six percent of the samples had at least one residue above the respective limit of detection. One hundred residues were found in soil, 112 in water, 99 in sediments, 78 in crops, 76 in outdoor air, and 197 in indoor dust. The number, levels, and profile of residues varied between farming systems. Our results show that non-approved compounds still represent a significant part of environmental cocktails and should be accounted for in monitoring programs and risk assessments. The hazard profiles analysis confirms the dominance of compounds of low-moderate hazard and underscores the high hazard of some approved compounds and recurring "no data available" situations. Overall, our results support the idea that risk should be assessed in a mixture context, taking environmentally relevant mixtures into consideration. We have uncovered uncertainties and data gaps that should be addressed, as well as the policy implications at the EU approval status level. Our newly introduced indicator can help identify research priority areas, and act as a reference for targeted scenarios set forth in the Farm to Fork pesticide reduction goals.

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3.10.P-Th160 Assessing the Environmental Hazard of Synthetic Phenolic Antioxidants

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Synthetic phenolic antioxidants (SPAs) are contaminants of emerging concern due to their extensive use in a wide range of applications, e.g., as preserving agents in plastics, fire retardants, synthetic fibers, fuels, and even foods. As a result, SPAs are now frequently detected in various environmental matrices, including natural water bodies, sediments, air or biota. Although prior studies have shown that certain SPAs and their transformation products can exhibit toxic effects in living organisms, data on their environmental fate and (eco-)toxicity remains sparse for a majority of compounds in this diverse and large class of substances.

This project aims to address this knowledge gap by investigating key environmental hazards of SPAs, namely persistence and (eco-)toxicity. As a first step, we assess the persistence of a broad range of SPAs as well as the formation of transformation products (i.e., ~100 substances), using activated sludge biotransformation assays. In this context, our goal is to streamline biodegradation experiments so that they are rapid yet robustly predictive of the compounds tendency to be biotransformed by complex environmental microbial communities, allowing for more efficient data collection for persistence assessment. Besides screening a large number of substances and developing a targeted LCMS analytical method, we will use these experimental systems to further explore a recently proposed alternative hazard assessment concept i.e., the cumulative and persistent toxicity equivalents (CTE/PTE) concept. The CTE/PTE concept couples biotransformation experiments with animal-free in vitro bioassays to test different cellular toxicity pathways so that key hazards of parent compounds and transformation products can be detected and quantified.

Our findings will provide critical data for environmental hazard assessment of SPAs. In addition, the tested workflows and optimized analytical method may eventually be integrated into the early stages of chemical development process for new emerging SPAs, thus providing essential information for the design of safe and sustainable chemicals.

3.10.P-Th161 Rapid Antimony Speciation in PET Additives Using Frontal Chromatography-ICP-MS: A New Approach for Environmental Risk Assessment

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Antimony (Sb), used as a catalyst in polyethylene terephthalate (PET) production, poses significant environmental and health risks due to its potential leaching into water sources. While PET commonly contains hundreds of mg/kg of Sb, current methodologies focus solely on total Sb content, overlooking the critical need for speciation of Sb(III) and Sb(V). This distinction is essential, as Sb(III) is more toxic and carcinogenic than Sb(V). Addressing this gap is vital for accurate risk assessment, especially as PET use continues to grow worldwide.

We present a novel, rapid method for Sb speciation using Frontal Chromatography coupled with Inductively Coupled Plasma Mass Spectrometry (FC-ICP-MS), optimized for analyzing Sb leaching from PET additives. The method utilizes a low-pressure column with a strong cation-exchange resin and nitric acid (HNO₃) as the eluent, allowing for efficient separation and detection of Sb(III) and Sb(V). Through systematic multivariate optimization, we achieved an exceptionally fast analysis time of 150 seconds and a detection limit below 1 ng/kg for both Sb species.

The application of this method to a variety of PET samples, including colored, virgin, recycled, and environmental plastics, revealed significant variability in Sb species migration. Aging and manufacturing processes notably influenced leaching behavior, with environmental samples showing the highest rates of Sb release. Across all samples, Sb(V) dominated the leachates, but Sb(III) levels were found to increase in naturally weathered plastics, indicating a shift in speciation over time.

Our FC-ICP-MS approach offers substantial advantages over traditional techniques, including faster analysis times, lower detection limits, and simpler instrumentation. The method's sensitivity and reliability make it a powerful tool for environmental monitoring and regulatory purposes. Additionally, its alignment with green analytical chemistry principles underscores its potential for widespread adoption in environmental health assessments, providing critical insights into Sb contamination and its ecological impacts.

3.10.P-Th162 How Common Oyster Mushroom Can Assist in Pharmaceutical Removal

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Emerging contaminants (EC), including pharmaceuticals, are insufficiently removed by conventional wastewater treatment processes, posing a significant risk to quality and safety of water resources.

Effluents from wastewater treatment plants (WWTPs) are a primary source of EC pollution in inland waters, underscoring the urgent need for efficient removal technologies. White-rot fungi, through a process known as mycoremediation, offer a promising bioremediation strategy due to the broad-spectrum activity of their ligninolytic enzymes, which can degrade a wide array of ECs.

This study explores the use of white-rot fungi, *Pleurotus ostreatus* M2191, for the bioremediation of pharmaceuticals through mycoremediation in wastewater effluent. Fungal pellets cultivated with Kraft lignin induced significant laccase enzyme production, with activity levels reaching up to 360 U/L. After 48 hours of fungal growth, pharmaceuticals (clarithromycin, sertraline, and venlafaxine) were introduced individually and as a mixture. Following 24 hours of exposure, fungal treatments demonstrated notable reductions in pharmaceutical concentrations, particularly for sertraline. Biosorption and extracellular degradation by laccases and other enzymes were identified as primary mechanisms of pharmaceutical removal, with biosorption favored for hydrophobic compounds. Additionally, the formation of transformation products (TPs) was evaluated using suspect screening, neutral loss, and common mass search, highlighting the role of white-rot fungi in environmental bioremediation and TPs formation.

This research supports the potential of fungal treatment for removing pharmaceuticals from water, offering insights into the mechanisms and efficacy of mycoremediation.

3.10.P-Th163 Investigating Quaternary Ammonium Compounds (QACs) Throughout Wastewater Treatment Plants and Receiving Waters

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Quaternary ammonium compounds (QACs) are a group of chemicals used in a wide range of consumer, agricultural, and industrial products, including disinfectants. Due to the COVID-19 pandemic, use of disinfectants has increased significantly since March 2020, increasing loads to wastewater plants and the environment. QACs have been shown to impact the development of antibiotic resistance and may be toxic to aquatic life. Thus, it is important to study the increasing use of disinfectants in the environment.

Quarterly influent, effluent and biosolid samples were taken from 10 wastewater treatment plants with varying sizes and treatment methods in Minnesota, Wisconsin and California, USA beginning in June 2021. At two plants, samples were taken along the treatment train to establish a mass balance during treatment. Surface water and surface sediment samples were also collected approximately a kilometer downstream of the wastewater discharge sites. These samples were analyzed for 25 QACs by solid phase extraction followed by analysis by LC-MS/MS. While >90% of QACs are removed during wastewater treatment, these chemicals are present in such high concentrations in influents that low µg/L

concentrations are still present in effluents. Additionally, biosolids have high levels of QACs. Because biosolids may be land-applied, this presents a route for the release of QACs to the environment and additional QACs could wind up in surface water and sediments through run off and leaching. QACs were present in high ng/L concentrations in the surface water collected, showing that effluent contributes a substantial amount of QACs to the environment

3.10.P-Th164 Chemical Pollution in Stormwater Ponds. Suspect Screening of Organic Micropollutants in Impacted Reservoirs in a Swedish Context

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Street run-off stormwater is often a hotspot for a wide range of organic pollutants as a result of traffic, pest control, road wear, buildings and other human activities. Although most organic micropollutants occur in low concentrations, many of them are highly toxic and, thus, even low concentrations might threaten the environment by harming aquatic organisms. Also, human health can be affected since, in Sweden, the vast majority of drinking water is produced from surface water.

In this work, we have investigated both influent and effluent water streams from two stormwater ponds impacted by, among others, heavy traffic and residential and industrial areas. Additionally, in case of technical failures, one of the ponds gets impacted by municipal wastewater as a nearby pump station for

sewage water directly discharges untreated wastewater to the pond during such operating conditions. Outlet streams of both stormwater ponds, eventually, mouth in Lake Mälaren (Sweden), which serves source water for drinking water production for 2 million people. Thus, it is of utmost importance to be well informed about the impact from inflowing contaminated water.

To investigate the impact of hydrological events on the fate of organic micropollutants in the ponds, water samples were collected both at dry periods (base flow) and rain events using flow proportional sampling. Mixed-mode solid-phase extraction (SPE) and ultra-high pressure liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS) were used for wide-scope screening of organic micropollutants of the samples permitting to expand the chemical coverage in comparison with conventional sample analysis strategies. Data revealed the presence of a large set of chemicals resulting from different human activities in both the influent and effluent streams from the stormwater pond. In total, near 70 organic micropollutants were identified, being vehicle related compounds and pharmaceuticals the most frequently detected compounds.

In this communication, we aim at shedding light into the pollution of stormwater ponds and its implication to the surrounding water bodies in a Swedish context.

3.10.P-Th165 Assessing Nanocarriers for their Environmental Risks

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Nanotechnology is nowadays considered a key technology of the 21st century, representing an umbrella for a multitude of products and processes rather than a single technology or application. It is simultaneously considered an "emerging technology" as well as an "emerging issue of environmental concern. One innovative technology are Nanocarriers or Nanocarrier systems (NCS). These NCS are innovative transport- and encapsulation systems that enable targeted delivery and release of active ingredients (AI) at a designated site. These systems achieve higher efficiency of the AI at the target site and therefore require a lower total amount used compared to conventional products. Despite their potential advantages, NCS might have the potential to become contaminants of emerging concern as there is a lot of uncertainty regarding their possible environmental risks. Their complexity, the variety of NCS formulations, and the missing definition and adequate testing and assessment strategies hamper proper risk assessment. Our project focuses on whether and to what extent existing guidelines like the OECD TG 318 can be adapted to assess the mobility, release and biodegradation of NCS under environmentally relevant conditions. Which analytical methods should be used and combined to assess the mobility and degradability of the nanocarrier and the (un-)intended release of the active ingredient in freshwater systems? As there is currently little information on the mobility of NCS in the environment and no harmonised methods to determine them, our research project aims to investigate appropriate methods for selected types of NCS to test their environmental behaviour at lab scale. We, therefore, developed a working definition and systematic categorisation of NCS based on size and function. Furthermore, three representative nanocarrier types are undergoing tests on their potential mobility under different environmental conditions (electrolyte composition, Suwannee River NOM extract content, pH, and temperature). The NCS are loaded with traceable fluorescent molecules, which may be released under changing environmental conditions. Fluorescence spectroscopy, UV-VIS and dynamic light scattering (DLS) are applied to test the nanocarriers dispersion and colloidal stability. The test protocol is inspired by OECD test guideline No. 318 for testing the dispersion stability of nanomaterials under simulated environmental conditions and is modified towards analysing NCS.

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3.10.P-Th166 Analysis of Phthalate Esters, Alternative Plasticizers, and Organic Phosphorus Flame Retardants in 46 Recycled Plastic Pellets

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Introduction

In a recycling-focused society, increasing the use of recycled plastics is essential. However, the safety of recycled plastics remains unclear. This study analyzed recycled plastic pellets made from polyethylene and polypropylene for phthalate plasticizers, alternative plasticizers, and organic phosphorus flame retardants.

Methods

The concentrations of these substances were measured in 44 types of recycled plastic pellets from 10 recycling plants in Japan. Approximately 200 mg of recycled plastic pellets were weighed and placed in a high-pressure container with 10 mL of cyclohexane, then heated to 150 °C for 4 hours to resolve the resin and extract the components. After centrifugation, the supernatant was filtered, and 1 mL of the filtrate was diluted with methanol to 10 mL to reprecipitate the resin. This mixture was then centrifuged and filtered again to remove the reprecipitated resin. The liquid was concentrated to 1.5 mL using nitrogen blowdown at 30 °C, after which internal standards were added, and the samples were analyzed by gas chromatography-mass spectrometry (GC-MS).

Results

The main plasticizers detected were diethylhexyl phthalate (DEHP) in 75% of the samples (N.D. (<0.1) to 54.5 µg/g), diisononyl phthalate (DINP) in 20% of the samples (N.D. (<0.01) to 33.3 µg/g), diisononyl adipate (DINA) in 41% of the samples (N.D. (<0.01) to 181 µg/g), tributyl acetyl citrate (ATBC) in 75% of the samples (N.D. (<0.02) to 60.8 µg/g), and diethylhexyl terephthalate (DEHT) in 86% of the samples (N.D. (<0.01) to 9.77 µg/g). The main organophosphorus flame retardants detected were tris (2-chloroethyl) phosphate (TCEP) in 39% of the samples (N.D. (<0.002) to 4.23 µg/g) and tris (1-chloro-2-propyl) phosphate (TCPP) in 68% of the samples (N.D. (<0.02) to 0.694 µg/g).

Discussion

In all of the recycled plastic pellets analyzed, the concentrations of the substances were all more than one order below the maximum allowable content (0.1%) of the four phthalates (DEHP, di-n-butyl phthalate (DnBP), benzyl butyl phthalate (BBP), and di-iso-butyl phthalate (DiBP)) specified in the RoHS Directive. In addition, the total concentration of the four phthalates restricted in toys and other products under the REACH regulations was also more than one order below the maximum allowable content (0.1%). Based on these findings, there are little safety concerns regarding these substances in the recycled plastic pellets in the plants measured in this study.

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3.10.P-Th167 Real-time Monitoring of 16 Pharmaceuticals in Wastewater using Automated On-Site SPE and LC-MS/MS Methods

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This study aimed to monitor 16 pharmaceuticals in the final effluent of a wastewater treatment plant in South Korea, utilizing automated on-site sampling and solid-phase extraction (SPE) combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Automated online solid-phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods exist, but the equipment is expensive, and there are no online systems that can be installed in the field for sampling. Pharmaceutical products (16 types) include acetaminophen, atenolol, caffeine, carbamazepine, diclofenac, ibuprofen, iopromide, lincomycin, naproxen, paraxanthine, propranolol, ranitidine, sulfamethoxazole, trimethoprim, and triclosan, and are substances detected in South Korea. In this study, the SPE system consisted of four components (Sampling, SPE, Extraction, and Injection) and was designed to achieve a 500-fold concentration. Considering 500-fold concentration, the lowest detection limit is 2 ng/L, although it varies depending on the substance. Optimization of SPE and LC-MS/MS under a variety of conditions for field samples improved sensitivity and selectivity. SPE cartridges are automatically washed and prepared to remove residual substances and effectively capture the analyte. For samples with complex matrices, such as wastewater, optimizing the SPE process required careful attention to the washing step, which removes interfering matrix components, and the cartridge drying step. Since the optimal pH conditions differ depending on the chemical properties of each pharmaceutical ingredient, the recovery rate and sensitivity for each ingredient were evaluated under various pH conditions. To find the optimal pH conditions, the pretreatment process was analyzed at various pH (3, 7, 10), and the recovery was evaluated using internal standards of carbamazepine-d10, ibuprofen-d3, atenolol-d7, sulfamethazine-13C3, sulfamethoxazole-d4 and trimethoprim-d9. Interestingly, only at pH 10 was the recovery of atenolol high at 85–110%. In this study, we systematically analyzed the effect of various conditions on the extraction efficiency of pharmaceutical ingredients during the SPE pretreatment process. This development enables real-time monitoring of pharmaceutical products in the field, and provides reliable data even from complex matrices of wastewater samples.

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3.10.P-Th168 Analysis of Aromatic Amines and Their Transformation Products in Dust from Five Different Indoor Environments Representing Diverse Source Profiles

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Aromatic amines (AAs) are found in a wide range of products, including pharmaceuticals, pesticides, rubber, textiles, hair dyes, and plastic products, and are by-products in tobacco smoke and certain cooking activities. Several AAs and their transformation products are classified as substances of very high concern. However, limited data exist regarding the presence and behavior of AAs in indoor environments, where human exposure may be significant, given that people spend over 90% of their time indoors. Indoor dust is a critical environmental matrix in this context for human exposure to various chemicals. Furthermore, indoor environments may contribute to wastewater contamination, as chemicals are potentially transferred to wastewater treatment plants through laundry water and cleaning. This study evaluates the occurrence, composition profiles, and potential sources of emerging AAs and related transformation products within dust across indoor environments with specific AA source profiles.

Dust sampling was conducted in five types of indoor spaces: a hairdresser, a restaurant kitchen, a smoking pub, a smoking home, and a non-smoking home. Repeated samples of settled dust were collected on quartz fiber filters over a six-week period using a vacuum cleaner equipped with a specialized sampling head. Wet wipe samples were also taken from elevated surfaces to complement dust sampling. An ultrasonic extraction method was developed to recover target analytes (primary AAs) from the dust samples, with methyl tert-butyl ether selected as the extraction solvent. AAs were consistently detected across the indoor spaces, with levels and composition profiles driven by the major sources in each type of indoor environment. The findings underscore the importance of indoor dust as a key environmental matrix for assessing exposure to AAs and other emerging contaminants and emphasize the need for further research on indoor sources of AAs, particularly to better evaluate occupational exposures and better characterize their pathways from indoor environments into surface waters via wastewater. The findings contribute to a better understanding of exposure from indoor sources and the tracking of high-concern substances that are important for both human health and environmental safety.

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3.10.P-Th169 Wastewater Pollution in Yucatan's Karstic Aquifer: Caffeine and Bacteria in Monitoring Wells of the Yucatan Peninsula

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In the Yucatan Peninsula (YP), the karst aquifer is associated with geological formations such as fractures and caverns, which give the soil great porosity and permeability. The aquifer is recharged and groundwater is transported to the coasts through the karst, processes that are fundamental in the water dynamics of the region. However, these same characteristics that allow the retention of large volumes of water also make the aquifer extremely vulnerable to contamination by wastewater. This vulnerability is aggravated by the intensification of human activity and the lack of adequate infrastructure for wastewater treatment, affecting the quality of water available to the population, a situation of great concern because groundwater is the only source of fresh water available to people.

In this study, the presence of caffeine in monitoring wells was evaluated. Caffeine can be used as a chemical tracer of the presence of human-origin wastewater in the aquifer, since the YP does not have a coffee industry, but the population consumes products containing caffeine (drinks, drugs, food); the caffeine consumed is excreted through urine and its final destination is wastewater. Likewise, the presence of fecal coliforms and enterococci was determined, in order to contrast the information provided by both indicators regarding contamination of human origin (non-specific biological tracer vs. chemical tracer). Water samples were collected in May 2024 (dry season) in 16 monitoring wells at two depths in the three states that are part of the YP: three wells in Campeche; one cenote and nine wells located in the northwest

area of the state of Yucatán, and three wells in Quintana Roo. Caffeine was determined by solid phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS). Bacteria were determined by membrane filtration using selective culture media. There was fecal contamination in 14 of the 16 sites analyzed, either by enterococci and/or fecal coliforms. Caffeine ranged from 10.6 to 2381.5 ng/L and it was detected in 100% of the wells at both depths, with higher concentrations at the surface than at mid-depth (except in the cenote); a general trend of increasing caffeine levels was also observed at the sites closest to the coast. The entry of untreated human wastewater was verified by the presence of faecal bacteria and caffeine. Detection of caffeine also indicates that there may be other compounds of emerging concern that can affect humans and biota.

3.10.P-Th170 Dissipation of Organic Micropollutants Originating from Sewage Sludge in Three Soils

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Sewage sludge from wastewater treatment plants, which are often supplied to the soil as a source of organic matter and other nutrients, can be contaminated with many micropollutants. These substances can then be taken up by plants, or they can migrate within the vadose zone and subsequently contaminate groundwater. The availability of these compounds for plants and their further spread in the environment depends on their sorption and stability in the soil environment. The persistence of substances in the soil is usually investigated under laboratory conditions, when individual compounds are applied into the soil and then the residual concentrations are analyzed over time. However, the dissipation of substances can be influenced by many factors, such as their initial concentration or the presence of other micropollutants and other substances in the contamination source. Therefore, this study focused on the analysis of the dissipation of selected sixteen micropollutants (including both parent compounds and metabolites), which were presented in sludge from a wastewater treatment plant, after its application into three different soils. In addition, the resulting dissipation trends were compared with the results of standard dissipation experiments for seven individual compounds applied into the same soils and carried out under identical conditions, which were published by Menacherry et al. (2023, <https://doi.org/10.1016/j.jhazmat.2023.132143>). For some compounds (1-methyl-1H-benzotriazole, lamotrigine, venlafaxine, and sertraline), the dissipation profiles were in both cases similar. On the other hand, in some cases, faster dissipation was found when the compound was applied individually (valsartan and atorvastatin), or when it was applied through sewage sludge (telmisartan).

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3.10.P-Th171 Biodegradation of Sulfonamides and Fluoroquinolones in Domestic Sewage using an Anaerobic Fixed-Bed Biofilm Reactor

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Among emerging contaminants, antibiotics have particular significance, since they can modify the microbial community, promoting antimicrobial resistance. Fluoroquinolones and sulfonamides are two important classes of antibiotics widely used in human and veterinary medicine worldwide. In this work, the removal of 7 antibiotics frequently detected in urban wastewater was evaluated in an anaerobic fixed-bed biofilm reactor (AnFBBR) treating domestic sewage during 60 days. The antibiotics consisted of 3 sulfonamides sulfamethoxazole (SMX), sulfadimethoxine (SDX) and sulfamerazine (SMZ); and 4 fluoroquinolones ciprofloxacin (CIP), pefloxacin (PEF), enrofloxacin (ENR), and ofloxacin (OFL). The domestic sewage was collected directly from the sewer pipes located near the Environmental Engineering campus of University of São Paulo (São Carlos, SP, Brazil). The bioreactor's performance was monitored through physical-chemical analyses: pH, alkalinity, volatile acids, chemical oxygen demand (COD), biogas composition (methane and carbon dioxide), and antibiotics. The antibiotics concentration was determined by using a column-switching online solid phase extraction coupled to a liquid chromatography/tandem mass spectrometry (SPE-LC-MS/MS) method - Chromatograph Agilent 1200 LC series and hybrid triple quadrupole-linear ion trap mass spectrometer AB SCIEX QTRAP 5500. A mixed solution of internal standards (SMX-C13, SDX-C13, CIP-D8, ENR-D5) was used to quantify the analytes and correct for matrix effects. The sewage had the following physical-chemical characteristics: COD = 525 ± 27 mg L⁻¹; pH = 7.47 ± 0.17 ; alkalinity = 172 ± 16 mg L⁻¹. The results indicated high performance

of the bioreactor in biodegrade organic matter, with COD removal efficiency = $87 \pm 3\%$, and methane content in the biogas $> 70\%$. The antibiotic removals were: SMX = $83 \pm 8\%$, SDX = $22 \pm 25\%$, SMZ = $55 \pm 24\%$, CIP = $56 \pm 21\%$, PEF = $80 \pm 10\%$, ENR = $82 \pm 9\%$, OFL = $73 \pm 6\%$. SMX, PEF, ENR, and OFL were the most easily biodegradable, while SMZ and CIP showed moderate biodegradation, and SDX showed high recalcitrance. AnFBBR proved to be feasible in removing antibiotics from sewage. However, complementary technologies that can be coupled to the AnFBBR should be further implemented in order to mitigate the effects of antibiotics on the environment, especially the spread and development of antimicrobial resistance genes.

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3.10.P-Th172 Toxicity of Newly Synthesized Ionic Liquids Based on the Acute *Daphnia magna* Immobilization Test

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Over the past few decades, the scientific community has been turning its attention to the potential widespread use of ionic liquids (ILs). Due to the non-volatility and relatively low toxicity of certain ionic liquids, their potential as a less toxic solvent has been recognized. However, the lack of data on a number of properties of ILs directs current research towards the assessment of their ecotoxicological effects. It has been shown in previous researches that combining different cations and anions affects the toxicity of ILs, and also that protic ILs and aprotic analogues show differences in their toxicity to organisms. Standard toxicity tests are suitable tools for the ecotoxicological characterization of various chemicals and may contribute to the assessment of the toxicity of ionic liquids, as well.

This study includes the analysis on seventeen newly synthesized protic ionic liquids that have 2-hydroxy-1-propylammonium, 1-hydroxy-2-methyl-2-propylammonium or triethanolammonium as cations, while anions are residues of various acids. To contribute to the knowledge about their toxicity, standard acute immobilization tests on *Daphnia magna* were carried out and EC50 values for all ILs were determined. The toxicity of the tested ILs was discussed in the context of experiments done in this study and a comparison of effects of the same ILs on different test models based on previous research. Based on the results of tests with *D. magna*, none of the ionic liquids proved to be toxic, one IL was categorized as harmful, while the other ILs showed no harmful effects. Taking into account the EC50 values from other tests, the *D. magna* tests showed a higher sensitivity to the investigated ionic liquids.

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3.10.P-Th173 Water Treatment Processes: Identification and Quantitation of Transformation Products Formed in Test Systems

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A recent European Food Safety Authority (EFSA) guidance document describes experimental methods for the identification of transformation products of active substances and their metabolites formed during water treatment processes. These processes include chlorination, chloramination, oxidation, ozonolysis, and ultraviolet light (UV) treatment. Here, we present results of experimental implementation of these processes carried out with a series of test substances.

Experiments were carried out in a standard water with addition of organic carbon obtained from the Suwannee River as defined in the guidance document. Chlorination with sodium hypochlorite was carried out at three different pH levels (6.5, 7.5, and 8.5). Monochloramine was prepared from sodium hypochlorite and ammonium chloride to carry out the chloramination experiments. For the oxidation process, an aqueous chlorine dioxide solution was obtained by addition of sulfuric acid to a solution of sodium chlorite.

In all of these experiments, the concentration of reagent in the test system was determined using the colorimetric N,N-diethyl-p-phenylenediamine (DPD) method to ensure that the requirements of the guidance document were met.

Ozonolysis was carried out by treating the test substances with a freshly prepared solution of ozone in

water, while the UV process was performed in an irradiation chamber fitted with a medium pressure UV lamp allowing control of the UV dose to the test solution.

Liquid chromatography coupled with mass spectrometry (LC/MS) was used to analyse the test substances and transformation products formed in these processes. Identification of the transformation products was carried out using high resolution-accurate mass spectrometry (HRAM) with data analysis performed using a non-target screening workflow, while quantitative analysis was performed by HRAM or multiple reaction monitoring (MRM) on a triple quadrupole mass spectrometer.

The results of these experiments are presented, with a discussion of challenges and suggested approaches to fulfilling the requirements of the guidance document.

3.10.P-Th175 Biocides in the Environment (BiU) - Advancements of the German Monitoring Database

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The Biocides in the environment (BiU) database was launched in 2023 with about 90,000 data entries, focusing on data from Germany, Austria and Switzerland, with the aim to collect and make biocide monitoring data publicly accessible. Biocidal products have a widespread use especially in urban areas as insecticides, disinfectants and preservatives that can lead to unavoidable emissions into various environmental compartments. Monitoring data can be used to address potential risks and raise awareness of environmental impacts of biocides. In 2024 the German Environment Agency (UBA) started developments to identify further suitable data sources and to establish the framework for a half-automatable extension of the "BiU database so that biocide monitoring data from German-speaking countries can be regularly integrated from various external data sources in future. Potential data sources were checked using respective inclusion criteria such as usage rights as well as the effort of data extraction and were matched with a priority list of 290 biocidal active substances and transformation products. Out of 66 reviewed environmental monitoring data sources, only 8 met the inclusion criteria and were further used. In total measurement data of 98 biocidal substances and transformation products were identified and included in the BiU database predominantly from Germany (96.4 %). Most of the measurement data is available for surface water (61.7 %) and groundwater (36.6 %), whereas data for soil, sediments, suspended particulate matter, or biota is rare. First analyses of the collected data, which comprise around 800,000 identified measured values, were conducted and will be presented. Key questions addressed the most frequently detected substances and the potential relation to specific application areas. Data will be used for further assessments, for example to evaluate temporal trends of specific biocides in environmental compartments or to have a closer look at the occurrence of specific substance groups like highly toxic pyrethroids. However, findings indicate that data for some environmental compartments is insufficient for gaining an appropriate overview of biocide contamination. Further efforts are still necessary to measure and collect environmental data for biocides and to make these data publicly available.

3.10.P-Th176 Environmental Concentrations of Bisphenol A in North American Surface Waters and Sediments Over the Years of 2010 to 2022

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Bisphenol A (BPA), a high-volume industrial chemical in widespread commercial use for over five decades, is used in the production of numerous consumer products and materials. Due to increasing regulatory scrutiny on BPA and other bisphenols for their potential endocrine-disrupting effects, various proposals for regulatory measures have emerged.

Extensive reports document the presence of BPA in environmental media, with a primary focus on aquatic ecosystems. This study provides a comprehensive analysis of measured BPA concentrations in the environment from 2010 to 2022. Monitoring data from both freshwater and saline surface waters and sediments across North America were gathered from peer-reviewed literature and publicly available monitoring databases. To ensure consistency and reliability, data were rigorously evaluated using the Criteria for Reporting and Evaluating Exposure Datasets (CREED) framework, developed by a team of experts, through the auspices of SETAC, aimed at standardizing the assessment of chemical exposure data. Relevant and reliable data were consolidated into a database covering 39 North American states and territories. Detection limits varied among studies, with a notable portion of data reported below the limits of detection (LOD).

The dataset was statistically processed to account for censored data and differences in detection limits. Summary statistics were calculated with adjustments for sample size and variations in sampling locations.

Mean concentrations, standard deviations, and other summary statistics were generated for each environmental compartment and compared to analyses from earlier periods. BPA concentrations displayed significant spatial and temporal variability across states and territories, with reported levels spanning several orders of magnitude across environmental compartments. This study has provided an updated understanding of BPA presence in natural surface waters, built on previous analyses conducted from the 1990s to 2010, and aimed to establish a robust statistical foundation for assessing environmental exposures of BPA across North America.

3.10.P-Th177 Plastic Additives in Sediments of the Mero-Barcés River Basin (NW Spain): Monitoring and Environmental Trends

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Plastics may leach harmful chemicals, such as plastic additives and monomers, into the environment. Some of these additives are classified as toxic substances and are listed under REACH; therefore, they have harmful effects on the environment. Sediments are generally the sink for organic pollutants (especially apolar contaminants) and are used to monitor spatial and temporal trends of these substances in the environment. However, sediments can also be a source of contaminants due to their resuspension and remobilization.

In this work, levels of plastic additives, including plasticisers, flame retardants, antioxidants, UV filters, and antimicrobials in sediments of the Mero-Barcés River Basin (northwest Spain) were evaluated seasonally between winter 2023 and winter 2024 (6 sampling campaigns, 4 sites each). The Mero-Barcés River Basin encompasses the river basins of two rivers that supply water to the Abegondo-Cecebre reservoir, which provides drinking water to 500,000 inhabitants in the entire metropolitan area of A Coruña. The basin is of great ecological and environmental value. The extraction of 55 additives from the sediments was performed by sonication, with determination by PTV-GC-MS/MS.

The highest levels of plastic additives were detected in spring 2023 sampling, with plasticisers being the main family in almost all sampling campaigns. A hot spot was detected in MB6, located near the intake point of the drinking water treatment plant. Regarding the individual additives, TiBP, TBHQ and DEP were not detected in any sample above their quantification limits, whereas 2,6-DIPN, DINP, and Tinuvin 234 were detected in all samples. The average concentration of all additives in these sediments was low, with the highest average values remaining below 1 µg g⁻¹. For most additives, there was a considerable variation in concentration depending on the sampling site and campaign.

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3.11 Integrating Ion Mobility Separations with HRMS: Transforming Environmental Contaminant Identification and Quantification Workflows

3.11.T-01 Targeted and Non-Targeted Analyses of PFAS in Human Serum Samples Collected Yearly from 2003 to 2021

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Per- and polyfluoroalkyl substances (PFAS) are a class of man-made chemicals commonly found in consumer and industrial applications as additives and surfactants. Recent evidence has linked several PFAS to concerning health outcomes, such as decreased vaccine response and increased cholesterol levels. Consequently, regulations have been introduced to limit the production and use of these substances, primarily targeting "legacy" PFAS, which have been extensively used and characterized over several decades. However, "emerging" PFAS remain largely unexplored due to their unknown chemistries and environmental presences. This is due to the analytical complexity of studying these chemicals, with data analysis workflow developments needed for their discovery and evaluation.

In this study, we present a novel non-targeted workflow that implements FluoromatchIM coupled to proprietary feature processing algorithms designed to utilize the unique orthogonality provided by ion mobility for PFAS analysis. Using this workflow, we simultaneously analyze quantitative targeted data, and non-targeted evaluations for a unique sample set comprising human serum samples collected from 2003 to 2021 (n = 156). The study specifically sampled serum from people with a systemic rheumatic disease (a recent diagnosis of one of Systemic lupus erythematosus (SLE), Rheumatoid arthritis (RA), Systemic sclerosis (SSC), Amyopathic dermatomyositis (ADM) or Juvenile dermatomyositis (JDM)), along with two control types: a sibling of similar age and an unrelated healthy control. This design enabled a comprehensive study of the potential role of PFAS exposure to the onset of an autoimmune disease, with controls designed to account for the influence of genetics.

The time span of sampling also enabled an in-depth temporal study of changes in PFAS exposure and bioaccumulation legacy and emerging during a critical period in global and domestic fluorinated chemical manufacturing. Moreover, the study includes individuals from major metropolitan areas such as Washington, D.C., New York City, and the Durham-Raleigh area, allowing for analysis of regional differences in PFAS exposure. Our results reveal regionalized patterns in PFAS bioaccumulation, contrasting known, legacy, PFAS with emerging PFAS. The findings suggest that emerging PFAS display distinct regionalized trends while legacy PFAS do not, suggesting the potential for unique exposure pathways for emerging PFAS which are mostly unstudied to date.

3.11.T-02 Unveiling the PFAS Fingerprint in Biota Leveraging the Technique LC-VIP HESI(-)-TIMS-HRMS and Untargeted Workflows

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The widespread environmental pollution caused by long-chain poly- and perfluoroalkyl substances (PFAS), known as forever chemicals, is a well-recognized issue in the last decades which has prompted significant chemical regulatory action and a global shift toward alternative PFAS. PFAS identification presents significant analytical challenges, such as the vast number of individual PFAS compounds, along with their transformation products and inefficiencies in MS/MS spectra acquisition, especially in data acquired using DIA modes. Given these complexities, it is essential to employ analytical techniques capable of identifying not only well-known PFAS but also identifying the PFAS "chemical fingerprint" in the environment.

The primary objective of this study was to evaluate the capabilities of ion mobility spectrometry (IMS) in the established LC-HRMS workflows for the in-depth monitoring of PFAS in complex environmental matrices, such as biota. This was achieved by leveraging the cutting-edge technique RPLC-VIP HESI(-)-TIMS-HRMS and an efficient untargeted workflow, combining novel chemometric tools and extensive suspect lists.

A PFAS dedicated sample preparation protocol was carried out, before IMS-HRMS analysis, which was conducted using the optimized broad mass and mobility transfer data dependent acquisition mode PASEF. An extensive suspect list containing more than 4,900 PFAS was used for the annotation of the features. Each annotated feature was thoroughly evaluated based on the precursor ion's mass error, isotope profile, retention time, MS/MS spectrum, and CCS value matching. Thus, the ion mobility-derived CCS values further enhanced the identification confidence by serving as an additional identification criterion. Some features were annotated with more than one fluorinated compound. To address this, a detailed investigation of the MS/MS spectra matching was conducted. Ion mobility filtering contributed to cleaner MS/MS spectra, while the data-dependent acquisition mode, PASEF, provided extensive MS/MS spectra coverage, which is often a challenge in untargeted workflows. These approaches enabled the confident identification of PFAS congeners. Additional chemometric tools were employed to support the untargeted identification and semi quantification of fluorinated compounds and their transformation products. This comprehensive IMS-HRMS-based untargeted workflow significantly enhanced the identification confidence of PFAS in biota samples.

3.11.T-03 Multidimensional-Constrained Suspect Screening of Hydrophobic Chemicals Using Gas Chromatography-Atmospheric Pressure Chemical Ionization-Ion Mobility-Mass Spectrometry **Xiaodi Shi¹, Anna Sobek² and Jonathan P. Benskin³, (1)Stockholm University, Stockholm, Sweden, (2)Environmental Science, Stockholm University, Sweden, (3)Stockholm University, Sweden**

Suspect screening strives to rapidly monitor a large number of substances in a sample using mass spectral libraries. However, for hydrophobic organic chemicals (HOCs), these libraries are primarily based on low-resolution electron ionization mass spectra. To improve the efficacy of suspect screening, new libraries and workflows are required, leveraging the highly specific analytical data acquired by state-of-the-art

mass spectrometers. In this study, we established a new library for 1,590 suspect contaminants, including identity, exact mass, and a combination of measured and model-predicted values for retention time (RT) and collision cross section (CCS). The assessment using standards for 102 environmental contaminants ($1.44 < \log K_{ow} < 16.8$; $-9.31 < \log K_{aw} < 11.3$) demonstrated that the ALLCCS2 model overpredicted CCS values (a mean relative deviation $\pm$ standard deviation [SD] of $-7.03\% \pm 5.42\%$) for HOCs, while uncertainties associated with RT predictions (mean of 0.086 min) are comparable to those of RTs converted from literature retention indices (mean of 0.15 min). Thereafter, using gas chromatography-atmospheric pressure chemical ionization-ion mobility-high resolution mass spectrometry, a suspect screening workflow constrained by full scan mass spectrum, RT, CCS, and fragmentation mass spectrum, together with a continuous scoring system, was established to reduce false positives and improve identification confidence. The true positive rates were estimated to be 79% and 64% for fortified and NIST standard reference marine sediment samples, respectively, with all false positives attributed to suspect isomers, indicating that the method is highly specific. The application of the novel method to surface sediment samples from the Baltic Sea, a Norwegian lake, and the East Siberian Sea screened 54 suspect contaminants, among which 11 tentative chemicals of emerging Arctic concern (CEACs) were detected in the East Siberian Sea sediment. An additional 34 compounds were found in the Arctic, but not listed as CEACs. This study offers a new workflow for improved suspect screening of HOCs using multidimensional information, and highlights the need to enrich CCS databases and extend the applicable chemical space of current in silico tools to non-polar contaminants.

3.11.T-04 Uncertainty Estimation of Qualitative and Quantitative Ion Mobility-Mass Spectrometry Workflows

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Integration of ion mobility-mass spectrometry (IM-MS) in workflows combining identity confirmation and quantification demands for uncertainty estimation of both collision cross section (CCS) measurement as well as of quantification using integration of arrival time-filtered MS signals. The ISO/IEC Guide 98-3:2008 recommends a bottom-up approach which identifies all possible sources of uncertainty and estimates the total combined uncertainty by propagating the random error caused by the variation of different influence quantities mathematically, or with Monte Carlo simulations. The latter has been applied in this study to estimate the measurement uncertainty of DTCCSN2. The application of uncertainty propagation including the uncertainties obtained for calibration standards (DTCCSN2 reference standards) revealed uncertainty estimates of $4.7\text{--}9.1\%$ ($k = 2$) for measured values using an established single-field calibration approach. Together with laboratory data obtained under repeatability conditions of measurement the confidence of DTCCSN2 in the context with identity confirmation was found to be comparable to retention indices used in GC.

During the last decade qualitative workflows have been developed and established, but the complexity of 4D LC-IM-TOFMS data has thus far hindered establishing an automated quantitative workflow. In this work, we are assessing the capabilities of LC-IM-TOFMS for quantification of contaminants of emerging concerns in surface water. Aqueous standards and spiked surface water samples were measured using both an LC-TOFMS and a LC-IM-TOFMS method. For the LC-IM-TOFMS data, a workflow using the open-source software packages MS-DIAL and Skyline was successfully developed. For identification of possible sources of uncertainty, an Ishikawa-diagram was created and employed to set-up a model equation for absolute concentration values obtained with the two methods. Uncertainty estimation revealed comparable measurement uncertainties for the results obtained with the two methods.

DTCCSN2 obtained from transient signals are reliable identification points which are currently integrated into routine identity confirmation workflows. As EURACHEM's bottom-up approach allows estimation of uncertainty of DTCCSN2 and the uncertainty of quantitative workflows which integrate ion mobility, both strategies can be embedded into routine methods and considered for accreditation according to ISO 17025.

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3.11.P-Th184 One Step Closer to Accurate Quantification of Micro- and Nanoplastics Using Cyclic Ion Mobility Mass Spectrometry

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Micro- and nanoplastics (MNPs) have been recognized as an environmental threat and there is global concern about their presence in humans. To investigate human exposure and the toxicological effects of

MNPs, robust methods to identify and quantify these plastic particles are essential. Pyrolysis coupled to gas chromatography mass spectrometry (Py-GC-MS) is currently the method of choice to analyse MNPs in complex biomatrices.

A novel direction in this field is the use of cyclic ion mobility spectrometry (cIMS) hyphenated to high-resolution MS (HRMS). The improved separation and resolution that can be achieved with Py-GC-cIMS-HRMS provide additional identification confidence and reduce spectral background noise, which improves method specificity and limit of detection. However, this novel instrumentation requires renewed method optimization, as for example optimal quantification markers ions are different compared to traditional Py-GC-MS.

In this contribution, we used a non-target approach to identify MS features that uniquely correlate to the polymers PE, PP, PS, PET, PVC and PMMA. Peak picking and integration of the experimental data resulted in 35,365 MS features to which linear calibration models could be fitted. Based on each model's coefficient of determination (R^2), optimal markers for each polymer were identified. This non-target approach was then extended to visualize unique pyrolytic products for the different polymers as homologue series, which were found to be consistent with literature. Data-independent acquisition of tandem MS data was used to annotate highly-correlating features, providing increased identification confidence and insight into the pyrolysis and ionisation processes.

By employing an experimental design that varied the sample matrix and ion source humidity, the quantification performance of the method could be further improved. Of all polymers, pyrolysis products of PET and PMMA appear to be most influenced by these factors. We observed that markers show intensity fluctuation up to 3 orders in magnitude in blood compared to blank matrix. These effects may induce large quantification errors if not considered, as MNP quantitation typically relies on out-of-matrix calibration. Finally, it was found increasing ion source humidity can increase marker response factors up to 3 orders of magnitude, which provides a route towards increasing the limit of detection for MNP quantification with Py-GC-cIMS-HRMS.

3.11.P-Th186 Ion-Mobility Derived CCS-m/z Trendlines for Improved Annotation Confidence of Contaminants of Emerging Concern and their Biotransformation Products

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Ion mobility high-resolution mass spectrometry (IM-HRMS) derived collision cross section (CCS) values can serve as an additional identification parameter in suspect and non-target screening analysis (SSA/NTS) of contaminants of emerging concern (CECs) and their biotransformation products. The utilization of CCS data usually relies on the comparison of experimental datapoints with library values or predicted CCS. However, for the latter high deviations to experimental data (> 10%) have been described for many compounds. The presented studies aimed to utilize CCS-m/z trendlines (i.e., describing the relationship between the CCS value and mass-to-charge ratio) for the annotation of suspect CECs and their biotransformation products.

In a first step, indoor dust samples ($n = 46$) were collected from 40 different locations in Flanders, Belgium. Phthalates and quaternary ammonium compounds (QACs) were identified as two relevant contaminant classes showing high abundances and detection frequencies. Three phthalates and 21 QACs were identified at confidence level (CL) 1. Additionally, 6 suspect phthalates and 17 suspect QACs, were tentatively identified. However, none could be assigned with a CL better than 3. Therefore, the CCS values for the assigned suspects were compared with the m/z-CCS trendlines established from available reference CCS data. This approach allowed the identification of extensive homologue series for both classes. For all suspect CECs, the experimental CCS values fell within the 95th percent confidence interval calculated for the reference CCS-m/z trendlines, contributing significantly to the confidence of the compound annotation. On the basis of these examples, a discussion on an appropriate communication of the added identification confidence was conducted.

In vitro biotransformation products of three QACs, identified as the most abundant homologues in indoor dust in the abovementioned study, were studied after incubation with human liver microsomes. A total of 31 phase I metabolites were annotated, derived from 19 biotransformation. For each metabolite average CCS values ($n = 6$) were calculated. After comparing these values with CCS data for parent QACs, changes in CCS values following metabolism were characterized. These findings describe characteristic gaseous confirmation of QAC metabolites and are expected to facilitate metabolite annotation in future in vitro and in vivo biotransformation studies.

3.11.P-Th190 Structural Insights into Dimeric Ions of Perfluorocarboxylic Acids (PFCAs) via Ion Mobility Mass Spectrometry (IMS-MS) and In-Silico CCS Prediction

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Per- and polyfluoroalkyl substances (PFAS) are contaminants of emerging concern, with over 7 million compounds currently inventoried. This vast number of compounds necessitates the use of non-targeted screening methods capable of identifying them. In this regard, combining ion mobility spectrometry (IMS) with liquid chromatography (LC) and high-resolution mass spectrometry offers a powerful approach, adding a supplementary separation dimension and providing collision cross sections (CCSs) as an additional identifier based on gas-phase ion density. In LC-IMS-MS experiments, with negative electrospray ionization, perfluorocarboxylic acids (PFCAs), tend to form homodimeric ions ($[2M-H]^-$) in addition to the expected deprotonated ion ($[M-H]^-$). This phenomenon has been noted in our study using trapped IMS (TIMS) but has also been reported using drift-tube IMS (DTIMS) and traveling-wave IMS (TWIMS). We found that these dimeric ions can dissociate after mobility separation, generating the corresponding deprotonated ion ($[M-H]^-$). As a result, the mobilogram of the deprotonated displays several peaks for a single m/z (mass-to-charge ratio) value: one for the monomeric $[M-H]^-$ ion and another for the $[M-H]^-$ ion resulting from the post-IMS dissociation of the dimeric ion ($[2M-H]^-$). This phenomenon can negatively affect the sensitivity of the method and complicate the identification process. Moreover, limited information is available on the conformations these dimers might adopt. Beyond their analytical relevance, understanding the conformations of these dimers could provide insights into the environmental fate of PFAS or their potential biological interactions. The aim of our study was therefore to propose plausible conformations for PFCA dimers using a qualitative analysis of CCS- m/z trends, complemented by in-silico geometry optimizations and CCS calculations. The computational approach we developed predicted accurate CCS values for both monomeric and dimeric ions, revealing that, in dimers, the fluorinated chains are likely in close proximity. For further research, we wonder whether similar proximity occurs when dimers form with larger cations like Na^+ or K^+ . Furthermore, we observed that certain perfluoroether carboxylic acids predominantly form dimers. Future studies should explore the impact of oxygen atoms in their fluorinated chains on dimer structure.

3.11.P Integrating Ion Mobility Separations with HRMS: Transforming Environmental Contaminant Identification and Quantification Workflows

3.11.P-Th178 Target and Non-Target Screening of Environmental Contaminants During Water Reuse by Trapped Ion-Mobility

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The myriad of potential environmental contaminants remains a significant obstacle when analysing complex environmental samples such as waste- or drinking water, as selecting appropriate target compounds is challenging. In particular, known or unknown transformation products (TPs) necessitate the use of advanced analytical techniques for their detection. Here, trapped ion mobility (TIMS) coupled to traditional liquid chromatography-mass-spectrometry (LC-MS) creates new opportunities for advancing targeted and non-target analyses of these complex mixtures. Using ion mobility introduces a novel characterisation parameter, enhancing selectivity and sensitivity for isobaric and isomeric compounds. In this work, we show two application examples utilizing LC-TIMS-MS for the identification and analysis of chemicals of emerging concern (CECs) in the aquatic environment. In the first example, advanced treated wastewater intended for potable and non-potable reuse was analysed by target and suspect screening. The second example employed non-target screening using patRoön for the analysis of ground- and drinking water samples. Aquatic samples were extracted by solid-phase extraction (Oasis HLB or Chromabond HR-X), evaporated, reconstituted in methanol, and extracts were diluted 1:5 before analysis by LC-TIMS-MS. A wide range of 100 CECs and TPs covering pharmaceuticals, pesticides, flame-retardants, and per- and polyfluoroalkyl substances, were analysed for their extraction efficiency, chromatographic separation, their mobility and detection limit. The mobility of each compound was assessed using three different concentrations to build an internal library of CCS values and compared to available literature.

Inclusion of CCS for compound identification improved method sensitivity but CCS values for comparison are currently limited in the literature. Moreover, using CCS to identify isomers remains

difficult due to similar measured mobilities, as observed for the TPs of diclofenac, ibuprofen, or carbamazepine. However, the applied workflow allowed detection limits as low as 0.55 ng/L due to the clean-up of mass spectra. Preliminary non-target screening data show that filtering by CCS effectively prioritises features, highlighting the refinements of including mobility in non-target screening workflows. Overall, this work shows the advantages of LC-TIMS-MS for analysing CECs and TPs in aquatic samples while identifying critical quality factors for future improvement.

3.11.P-Th179 Application of Trapped Ion-Mobility Spectrometry for Environmental Contaminant Detection in Advanced Treated Wastewaters

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Trapped ion mobility spectrometry (TIMS) is an innovative technique that effectively separates ionized compounds based on their charge, shape, and size, facilitating the removal of co-eluting isomeric/isobaric species. TIMS is often combined with ultra-high performance liquid chromatography (UHPLC) and high-resolution mass spectrometry (HRMS) to enhance the reliability of chemical identification. This is achieved by translating TIMS data into collision cross-section (CCS) values. The CCS provides an estimate of the size of the ion's colliding sphere as it traverses the gas phase, serving as an extra parameter for chemical identification and enabling comparability of measured CCS values across different instruments. However, TIMS for the identification of environmental contaminants has been scarcely explored, especially in treated wastewater matrices. In this study, a total of 100 reference standards including pharmaceuticals, pesticides, and per- and polyfluoroalkyl substances were analyzed through targeted analysis in both positive and negative ion modes using UHPLC coupled with TIMS and time-of-flight mass spectrometry (UHPLC-TIMS-TOF-MS). The TIMSCCSN2 values of the reference standards were obtained, and the accuracy of these measurements was evaluated by assessing various software parameters. The average values were employed to critically assess previously reported data from different instruments, calibration workflows, and predicted values. These findings were then used to increase the sensitivity of the detection of these environmental contaminants after advanced wastewater treatments with ozonation, ceramic membrane filtration, and activated carbon using the CCS values associated to each analyte as a filter, thus reducing the interferences and background noise and allowing their detection at trace levels. The findings not only improve the identification of environmental chemicals in wastewater through targeted analysis but also contribute valuable data to the scientific community. This supports efforts toward standardization across laboratories and aids in reducing false positive identifications in non-targeted analyses.

3.11.P-Th180 An Advanced Analytical Methodology for the Simultaneous Monitoring of more than 2,500 Emerging Pollutants in Soils and Biodiversity Leveraging the Technique LC-TIMS-HRMS

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Environmental pollution remains a critical global challenge as terrestrial ecosystems are continuously exposed to a diverse and dynamically changing mixture of anthropogenic chemicals, commonly referred to as emerging pollutants. Systematic monitoring of these chemicals supports the European Commission's Chemicals Strategy for Sustainability, aiming to protect the environment, wildlife, and human health through a One Health approach. High Resolution Mass Spectrometry (HRMS)-based workflows are powerful tools for the simultaneous detection of emerging pollutants, covering a wide range of substances with diverse applications and physicochemical properties, including their metabolites and transformation products. Additionally, Ion Mobility Spectrometry (IMS) provides an additional dimension of separation, enhancing the identification of chemicals in complex matrices, such as biological organisms.

Within this context, the TerraChem project aims to develop, demonstrate, and apply a novel approach that integrates monitoring, environmental modeling, data management, analytical tools, and user guidance. The overall aim is to enhance the understanding of exposure of terrestrial biota across trophic levels to the universe of environmentally relevant anthropogenic chemicals and resulting damage on biodiversity and

ecosystem services.

In the framework, aiming to expand the chemical domain accessible to post-acquisition data treatment, generic sample preparation protocols were implemented to enrich final extracts with chemicals of varying physicochemical properties. The instrumental analysis was performed using LC-TIMS-HRMS. A comprehensive 4D target screening, utilizing a database of over 2,500 emerging pollutants, revealed that terrestrial wildlife is exposed to complex mixtures of anthropogenic chemicals. The results underpin the enhanced identification capabilities provided by the TIMS dimension within established LC-HRMS workflows, leading to increased confidence in the final identification of pollutants.

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3.11.P-Th181 Suspect and Non-Targeted Screening of Halogenated Contaminants in a Stranded Killer Whale (*Orcinus orca*) using GC-HRMS hyphenated with trapped ion mobility (TIMS)

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The killer whale (*Orcinus orca*) is likely the most contaminated marine mammal globally, facing extremely high levels of contaminants. Beyond well-known pollutants like legacy persistent organic pollutants (POPs), killer whale tissues likely contain various emerging and unknown contaminants, potentially leading to additional toxic impacts. In this context, this study aims to assess the advantages of using high-resolution ion mobility for the suspect and non-targeted screening of halogenated contaminants in the blubber of a stranded killer whale.

Approximately 70 mg of blubber were collected from a killer whale that stranded on the beach of De Panne, Belgium. Following a simple sample preparation, the resulting solution was analyzed using a TIMS TOFpro II mass spectrometer (Bruker, Bremen) equipped with a GC-APCI source for sample separation and ionization before IM-MS analysis.

Suspect screening was conducted for various classes of legacy (e.g., PCBs, PBDEs, OCPs) as well as emerging (e.g., PXBs) persistent organic pollutants (POPs). Overall, approximately 150 suspected POPs were tentatively confirmed, with the vast majority being PCBs (104 congeners identified). Comparison of experimental CCS with those available in our in-house database greatly contributed to the confident confirmation of these features, since CCS deviations were typically below 1%. For those features whose CCS was not present in the database, confident confirmation of the contaminant class could still be obtained by checking whether these belonged to the CCS vs m/z trendline of a specific class of halogenated POP.

For the non-targeted analysis, several compounds were identified as probable halogenated compounds, based on their mass defect and isotopic pattern, which are characteristic for halogen-bearing molecules. Here, the additional dimension of separation provided by the ion mobility was of great help in obtaining clean mass spectra. Indeed, filtering the data according to the ion mobility unable to get rid of most matrix background signal as well as other coeluting isobaric interferences, greatly improving the quality of the spectra and the identification process, especially for low signal intensity features.

Taken together, these preliminary results demonstrate the potential of using IM to enhance both suspect and non-targeted screening of halogenated contaminants in complex matrices.

3.11.P-Th182 GC-Ion Mobility-HRMS as a Novel and Powerful Alternative to Magnetic Sector MS for a Comprehensive Quantitation and Identification of Dioxins and Multiple Classes of POP

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For the past decades, GC-HRMS on magnetic sector instruments has been the main technique approved by the regulatory bodies for dioxin quantification, because of its high selectivity and sensitivity in food and environmental samples. Magnetic sector instruments are costly in acquisition and maintenance and require highly skilled personnel. Consequently, the obligation for applying GC-HRMS for dioxin analysis is a limiting factor for monitoring these toxic pollutants in many countries. Proposed here is a novel workflow

which combines a GC-APCI source for high sensitivity, trapped ion mobility (TIMS) as an orthogonal criterion for the dioxin quantitation, and a high-resolution, ultra-sensitive QTOF. One of the main benefits of this approach is that various classes of POP can be analyzed and quantified jointly with dioxins in a single GC run.

1 μ L of a sample containing the 29 known toxic congeners (dioxins, furans, PCB) was injected into the GC (35 min run, Restek 60 μ m $\times$ 0.25 μ m column). A GC-APCI source was coupled to an ion mobility QTOF (timsTOF Pro 2, Bruker) enabling fast and sensitive quantitative analysis of these different classes of POP in a single GC/HRMS run. Criteria for validation and quantification of compounds included mass accuracy, retention time, isotope pattern matching, MS/MS qualifiers and collision cross sections (CCS) from ion mobility filtering. Kendrick mass defect plots filtered specific compounds containing Cl or Br from the complete GC/MS chromatogram.

The capabilities of the combination of GC-APCI-Trapped Ion Mobility-HRMS for the confident and sensitive quantitation of POP in accordance with the performance criteria of the worldwide standard methods (EPA 1613B, EN 1948) for environmental and food samples were evaluated and validated. It is shown that GC-APCI-TIMS-HRMS is a valid alternative to traditional magnetic sector MS with the benefit of a higher analytical flexibility for more POP classes and the separation of isomers and isobars. All PCBs have been detected at an LOD of 10–20 ppt. The dioxins were detected down to levels of 25–125 ppt, depending on the individual compound. Examples of the analysis of real-life samples like rapeseed oil, milk fat, sludge extract and ash are presented.

3.11.P-Th183 Detection and Characterization of Environmental Contaminants by Direct Analysis in Real Time (DART) Mass Spectrometry Techniques

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PFASs or per- and polyfluoroalkyl substances is a widely used term for a broad family of chemicals that possess at least one fully fluorinated methyl or methylene group (CF₃ or CF₂, respectively). In most cases, these molecules are extensively fluorinated, with long aliphatic chains saturated with C–F bonds. PFASs have been commonly used in everyday life for almost a century, in products such as pharmaceuticals, pesticides, fabrics, nonstick cookware, adhesives, cosmetics, and even food packaging. Although these fluorinated substances may undergo chemical breakdown at their functional groups (typically carboxylic acid, sulfonic acid, or ether linkages), the high energy of the C–F bond (488 kJ/mol) leads to their endured persistence and inertness in the environment. If ingested, PFASs can interfere with biological processes causing various health ailments and diseases. Another class of environmental contaminants that can cause adverse health effects are para-phenylenediamine (PPD) antioxidant additives in commercial materials, such as automobile tires. Once released into water or air, these diamines are easily oxidized to quinones which are toxic. Here, we demonstrate a sensitive, mass spectrometry (MS) based analytical procedure for the detection and quantitation of such environmental contaminants. It involves the use of the DART ambient ionization method coupled to inline ion mobility (IM) separation and tandem mass spectrometry (MS/MS) characterization. This DART-IM-MS/MS approach proceeds with practically no sample preparation, requires very small sample quantity, allows for fast sample fractionation by collision cross-section in the IM dimension, and provides conclusive structural identification of the separated sample components based on mass accuracy and fragmentation pattern in the MS and MS/MS dimensions, respectively; these advantages will be demonstrated with the analysis of several PFAS and PPD compounds.

3.11.P-Th184 One Step Closer to Accurate Quantification of Micro- and Nanoplastics Using Cyclic Ion Mobility Mass Spectrometry

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Micro- and nanoplastics (MNPs) have been recognized as an environmental threat and there is global concern about their presence in humans. To investigate human exposure and the toxicological effects of MNPs, robust methods to identify and quantify these plastic particles are essential. Pyrolysis coupled to gas chromatography mass spectrometry (Py-GC-MS) is currently the method of choice to analyse MNPs in complex biomatrices.

A novel direction in this field is the use of cyclic ion mobility spectrometry (cIMS) hyphenated to high-resolution MS (HRMS). The improved separation and resolution that can be achieved with Py-GC-cIMS-

HRMS provide additional identification confidence and reduce spectral background noise, which improves method specificity and limit of detection. However, this novel instrumentation requires renewed method optimization, as for example optimal quantification markers ions are different compared to traditional Py-GC-MS.

In this contribution, we used a non-target approach to identify MS features that uniquely correlate to the polymers PE, PP, PS, PET, PVC and PMMA. Peak picking and integration of the experimental data resulted in 35,365 MS features to which linear calibration models could be fitted. Based on each model's coefficient of determination (R^2), optimal markers for each polymer were identified. This non-target approach was then extended to visualize unique pyrolytic products for the different polymers as homologue series, which were found to be consistent with literature. Data-independent acquisition of tandem MS data was used to annotate highly-correlating features, providing increased identification confidence and insight into the pyrolysis and ionisation processes.

By employing an experimental design that varied the sample matrix and ion source humidity, the quantification performance of the method could be further improved. Of all polymers, pyrolysis products of PET and PMMA appear to be most influenced by these factors. We observed that markers show intensity fluctuation up to 3 orders in magnitude in blood compared to blank matrix. These effects may induce large quantification errors if not considered, as MNP quantitation typically relies on out-of-matrix calibration. Finally, it was found increasing ion source humidity can increase marker response factors up to 3 orders of magnitude, which provides a route towards increasing the limit of detection for MNP quantification with Py-GC-cIMS-HRMS.

3.11.P-Th185 Are Collision Cross Section Values Comparable Across Different Ion Mobility Separation Instrumental Designs? An Interlaboratory Evaluation

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The hyphenation of ion mobility separation (IMS) to high resolution mass spectrometry (HRMS) has shown clear benefits for the identification of contaminants of emerging concern in complex environmental samples. IMS separates ions based on their shape, size and charge; and permits the measurement of collision cross section (CCS) values for the analyzed ions. This value gives an overall estimation of the size of the colliding sphere created by the ion when moving across a gas phase. However, the deviation error of CCS values for the different adducts, e.g., $[M+H]^+$, $[M-H]^-$, $[M+Na]^+$, etc., between different IMS-HRMS technologies and/or instrumental designs is not fully clear. In this NORMAN interlaboratory exercise, we aim at evaluating the comparability of the measured CCS values across different instrumental platforms, establishing a maximum CCS value deviation from third-party measurements as well as identifying relevant analytical metadata that should be shared alongside CCS values for enhanced data quality.

To this end, a total of 76 reference standards comprising pesticides, pharmaceuticals, personal care products as well as other organic micropollutants have been measured in 19 laboratories, each of them with their own in-house methods. The study yielded 6,675 CCS data points (2,916 for $[M+H]^+$, 620 for $[M+K]^+$, 1,520 for $[M+Na]^+$ and 1,619 for $[M-H]^-$). This CCS measurement dataset was analyzed to address critical questions regarding CCS repeatability, quantitative evaluation of the average expected error, investigation of protomer generation factors, and evaluation of the impact of different calibration methods and IMS technologies on CCS accuracy. The dataset served as a valuable resource for confronting analytical challenges related to CCS measurement variability and provides a basis for future standardization efforts across laboratories. Overall, with the increasing availability of online databases as well as studies reporting CCS values, the IMS community needs clear and robust evidence on the possibility of comparing their own data with third-party data as demonstrated in this study.

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3.11.P-Th186 Ion-Mobility Derived CCS-m/z Trendlines for Improved Annotation Confidence of Contaminants of Emerging Concern and their Biotransformation Products

Lidia Belova¹, Mikel Musatadi², Maosen Zhai¹, Maarten Roggeman¹, Giulia Poma³, Celine Gys¹, Paulien

Cleys¹, Fatima den Ouden¹, Patrick Berghmans⁴, Jan Peters⁴, Maitane Olivares², Alexander van Nuijs³ and Adrian Covaci¹, (1)University of Antwerp, Belgium, (2)University of the Basque Country, Spain, (3)University of Antwerp, Belgium, (4)Flemish Institute for Technological Research (VITO), Belgium Ion mobility high-resolution mass spectrometry (IM-HRMS) derived collision cross section (CCS) values can serve as an additional identification parameter in suspect and non-target screening analysis (SSA/NTS) of contaminants of emerging concern (CECs) and their biotransformation products. The utilization of CCS data usually relies on the comparison of experimental datapoints with library values or predicted CCS. However, for the latter high deviations to experimental data (> 10%) have been described for many compounds. The presented studies aimed to utilize CCS-m/z trendlines (i.e., describing the relationship between the CCS value and mass-to-charge ratio) for the annotation of suspect CECs and their biotransformation products.

In a first step, indoor dust samples (n = 46) were collected from 40 different locations in Flanders, Belgium. Phthalates and quaternary ammonium compounds (QACs) were identified as two relevant contaminant classes showing high abundances and detection frequencies. Three phthalates and 21 QACs were identified at confidence level (CL) 1. Additionally, 6 suspect phthalates and 17 suspect QACs, were tentatively identified. However, none could be assigned with a CL better than 3. Therefore, the CCS values for the assigned suspects were compared with the m/z-CCS trendlines established from available reference CCS data. This approach allowed the identification of extensive homologue series for both classes. For all suspect CECs, the experimental CCS values fell within the 95th percent confidence interval calculated for the reference CCS-m/z trendlines, contributing significantly to the confidence of the compound annotation. On the basis of these examples, a discussion on an appropriate communication of the added identification confidence was conducted.

In vitro biotransformation products of three QACs, identified as the most abundant homologues in indoor dust in the abovementioned study, were studied after incubation with human liver microsomes. A total of 31 phase I metabolites were annotated, derived from 19 biotransformation. For each metabolite average CCS values (n = 6) were calculated. After comparing these values with CCS data for parent QACs, changes in CCS values following metabolism were characterized. These findings describe characteristic gaseous confirmation of QAC metabolites and are expected to facilitate metabolite annotation in future in vitro and in vivo biotransformation studies.

3.11.P-Th187 Sample-Specific Machine Learning Modelling for CCS Prediction to Improve Compound Identification in Non-Target Analysis

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In current non-target mass-spectrometry (MS) experiments for environmental screening, more than 95% of measured spectra can not be correctly annotated. By leveraging machine learning (ML) modelling of additional analytical dimensions, the analyst can prioritize candidates through comparison of the prediction with the measured value. Most instruments in non-target analysis encompass a chromatograph as well as an MS, and some of them also feature a third separation dimension in the form of ion-mobility (IM). Although IM has been shown to be semi-orthogonal to MS and thus less informative than for example chromatography, IM instruments are capable of separating structural as well as geometric isomers and even diastereoisomers, making it a powerful complementary dimension. A typical ML workflow for collision cross-section (CCS) prediction relies on having the (putative) molecular structure of the compound in question to compute a molecular representation. To that end, experimental CCS databases covering different regions of the chemical space have been generated. Although CCSs are more reproducible than retention times, database merging still poses a problem difficult to tackle. On the other hand, training models on a sole database, that usually covers a smaller region of the chemical space, limits their application to certain samples. Our approach tries to tackle the applicability domain by building sample-specific models. ML will thus be trained on the most relevant data to the sample in question in terms of chemical space coverage. To do so, a molecular representation is inferred from each MS feature using ML. All representations are then used to train a CCS regression model. In a parallel way and independently to regression training, a candidate list is generated for each MS feature through available software. From each candidate structure, the same type of molecular representation as before is calculated and used to obtain a CCS value through the previously trained regression model. We will also implement probabilistic approaches to make statistical comparisons with the measured CCS value for candidate structure prioritization. The main challenge will be selecting a suitable molecular representation that (a) can be calculated from both the MS as well as from the structure and (b) can encode all the relevant information to predict CCS. We note that no stereochemistry will be involved. Its influence on CCS prediction is yet to be determined.

3.11.P-Th188 Overcoming Analytical Challenges in Xenometabolome Assessment - From Isomer Separation to Comprehensive MS/MS Coverage Combining LC-HRMS with TIMS-PASEF

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Xenometabolome characterization, encompassing the identification of biotransformation products (bio-TPs) modified by an organism in response to xenobiotic exposure, is essential for gaining insights into the mechanisms of toxicity and potential ecological impacts. Despite the technological advancements in LC-HRMS, the identification of bio-TPs remains challenging. A major challenge is the vast number of different biotransformation reactions, while some of them are still under investigation. On the other hand, even well-characterized reactions (e.g., hydroxylation) may lead to different isomeric bio-TPs. Sensitivity limitations can hinder low-abundance bio-TP detection, preventing a complete understanding of metabolic pathways. Finally, a crucial analytical challenge in the context of unknowns identification is the limited availability of experimental MS/MS spectra, highlighting the need for comprehensive data-dependent acquisition (DDA) modes.

To overcome the aforementioned challenges, this study presents a multidimensional analytical workflow integrating complementary chromatographic separations with trapped ion mobility spectrometry (TIMS), HRMS, and mobility-aligned DDA acquisition methods (Parallel Accumulation Serial Fragmentation - PASEF), to maximize analytical evidence generation. Zebrafish embryos exposed to benzotriazoles served as a case study, to demonstrate the workflow's effectiveness for bio-TPs identification.

The use of orthogonal separation techniques (RPLC, HILIC, TIMS) expanded the available analytical space, providing robust evidence for identifying both suspects and unknowns. TIMS dimension facilitated the separation of isomeric bio-TPs (OH-sulfate-MeBT) and reduced background interferences, leading to cleaner, more informative spectra. The improved S/N facilitated the detection of new low-abundance bio-TPs enabling a more complete xenometabolome assessment. Mobility-aligned DDA (PASEF) delivered extensive MS/MS coverage (>70% of precursors), capturing high-quality MS² data even for low-intensity precursors, which is essential for confident identification of unknown bio-TPs. In conclusion, the integration of TIMS and PASEF with RPLC/HILIC-HRMS establishes a data-rich workflow that facilitates xenometabolome characterization, achieving high-confidence identifications and addressing key analytical challenges.

3.11.P-Th189 Investigating the Effect of Combining Trapped Ion Mobility Spectrometry with HRMS and its Resulting Data Acquisition Modes in Untargeted Workflows for Identifying Emerging Contaminants

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Untargeted workflows using liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) play an important role in the identification of emerging contaminants (ECs). However, the effectiveness of untargeted workflows is influenced by the data acquisition schemes. This study investigates how incorporating trapped ion mobility spectrometry (TIMS) and Parallel Accumulation-Serial Fragmentation (PASEF) data-dependent acquisition (DDA) mode into LC-HRMS workflows impacts data quality and the identification of unknowns. TIMS separates ions in the gas phase based on their different size- and shape-to-charge ratio, while PASEF, enabled by TIMS, provides fast DDA MS/MS spectra without compromising sensitivity by synchronising the MS/MS precursor selection with TIMS separation.

To assess the impact of these technologies, standard solutions and spiked samples (blood serum, urine and wastewater) containing >200 emerging contaminants were prepared at different concentration levels and analyzed with and without TIMS dimension. The target compounds were treated as unknowns to evaluate the performance of the untargeted workflow. We specifically evaluated cases of missing MS/MS spectra, low-quality MS/MS spectra and co-fragmentation, across different acquisition modes. Moreover, we investigated the ability of TIMS to separate co-eluting isomeric/isobaric compounds, reduce background interferences, and enhance signal, facilitating the detection of low concentration analytes in complex matrices.

The addition of TIMS and PASEF in LC-HRMS significantly improved the identification of unknowns. TIMS dimension and mobility filtering increased sensitivity, leading to the detection of more features. PASEF acquisition provided a higher number of MS/MS spectra with improved quality, characterised by higher intensities and more fragments in some cases. This resulted in more confident compound identification, particularly in complex matrices (wastewater) and at lower concentration levels. Our

findings demonstrate that TIMS and PASEF enhance the performance of untargeted LC-HRMS workflows, by generating multidimensional high quality data, leading to more comprehensive and reliable identification of unknown ECs.

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3.11.P-Th190 Structural Insights into Dimeric Ions of Perfluorocarboxylic Acids (PFCAs) via Ion Mobility Mass Spectrometry (IMS-MS) and In-Silico CCS Prediction

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Per- and polyfluoroalkyl substances (PFAS) are contaminants of emerging concern, with over 7 million compounds currently inventoried. This vast number of compounds necessitates the use of non-targeted screening methods capable of identifying them. In this regard, combining ion mobility spectrometry (IMS) with liquid chromatography (LC) and high-resolution mass spectrometry offers a powerful approach, adding a supplementary separation dimension and providing collision cross sections (CCSs) as an additional identifier based on gas-phase ion density. In LC-IMS-MS experiments, with negative electrospray ionization, perfluorocarboxylic acids (PFCAs), tend to form homodimeric ions ($[2M-H]^-$) in addition to the expected deprotonated ion ($[M-H]^-$). This phenomenon has been noted in our study using trapped IMS (TIMS) but has also been reported using drift-tube IMS (DTIMS) and traveling-wave IMS (TWIMS). We found that these dimeric ions can dissociate after mobility separation, generating the corresponding deprotonated ion ($[M-H]^-$). As a result, the mobilogram of the deprotonated displays several peaks for a single m/z (mass-to-charge ratio) value: one for the monomeric $[M-H]^-$ ion and another for the $[M-H]^-$ ion resulting from the post-IMS dissociation of the dimeric ion ($[2M-H]^-$). This phenomenon can negatively affect the sensitivity of the method and complicate the identification process. Moreover, limited information is available on the conformations these dimers might adopt. Beyond their analytical relevance, understanding the conformations of these dimers could provide insights into the environmental fate of PFAS or their potential biological interactions. The aim of our study was therefore to propose plausible conformations for PFCA dimers using a qualitative analysis of CCS- m/z trends, complemented by in-silico geometry optimizations and CCS calculations. The computational approach we developed predicted accurate CCS values for both monomeric and dimeric ions, revealing that, in dimers, the fluorinated chains are likely in close proximity. For further research, we wonder whether similar proximity occurs when dimers form with larger cations like Na^+ or K^+ . Furthermore, we observed that certain perfluoroether carboxylic acids predominantly form dimers. Future studies should explore the impact of oxygen atoms in their fluorinated chains on dimer structure.

3.11.P-Th191 Comparison of Electrospray Ionisation with Unispray in Ion Mobility-High-Resolution Mass Spectrometer for Suspect and Non-Target Screening

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Ion mobility (IM) coupled with high-resolution mass spectrometry (HRMS) has become more and more popular for suspect and non-target screening (SNTS) of environmental samples, where ion mobility can increase the confidence in identification of unknown compounds. High mass accuracy measurements with HRMS facilitates the discovery of unknown and unexpected compounds in environmental samples. However, the downside is the lower sensitivity compared to targeted approach using low-resolution mass spectrometers. This can inhibit the detection of low-abundance compounds in SNTS. Unispray is a newer type of ion source which has been shown previously to yield a higher sensitivity for target analysis in comparison to the commonly used electrospray ionization (ESI). Our aim is to investigate the suitability of using Unispray for chemical screening in environmental matrices. For this purpose, the ion source parameters are optimized for both Unispray and ESI for a broad range of environmentally relevant compounds with different physio-chemical properties. Comparison will thereafter be made regarding their intensities, signal-to-noise, reproducibility, robustness, and precision, among others. Furthermore, the extent of in-source fragmentation and MS² spectra similarity are compared for the compounds. Lastly, samples with different matrices (drinking water, wastewater, and dust) will be used for matrix effect comparison as well as their suitability in SNTS based on performance on detecting unknown compounds.

To facilitate the SNTS, an in-house spectral library has been constructed with MS2 fragments from ESI for a wide range of different compounds suitable for its use in open-source data processing software. Additionally, the spectral library contains the CCS values, which can increase the confidence in the identification of compounds. Furthermore, a workflow has been developed to process high-resolution ion mobility data with open-source software. From our preliminary results, Unispray seems well-suited to be applied for SNTS since it in general shows 3-15 times higher signal intensities, 2-17 times higher signal-to-noise, and 2-17 times lower limits of detection compared to ESI, for the measured compounds. Hence, Unispray shows good promise to better detect lower abundance compounds in real samples than ESI.

3.11.P-Th192 Exploring the Integration Benefits of Ion Mobility Mass Spectrometry (IMS) and MS2 Analytical Workflows with MALDI-HRMS for Small Molecule Analysis; Zebrafish Exposed to Xenobiotics as a Case Study

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Matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a powerful analytical tool, traditionally utilized for the analysis of large molecules like proteins and glycans. However, its application in small molecules analysis can be challenging due to interfering matrix-related ion in the lower m/z mass range. Despite that, the growing interest in omics approaches for unveiling toxicity and disease pathways, especially with the integration of imaging techniques, has increased the relevance of MALDI in small molecules analysis. Coupling ion mobility spectrometry (IMS) with high-resolution mass spectrometry (HRMS) techniques, adds a size-based separation dimension, enabling the separation of isomeric and isobaric species. This is particularly relevant for omics approaches, such as lipidomics, where identifying structurally similar molecules is crucial. IMS also provides collision cross section (CCS) values, further enhancing the confidence and specificity of analyte identification. Therefore, the integration of IMS with MALDI, promises significant advancements in small molecules analysis. This study explores the benefits of integrating trapped ion mobility (TIMS) with MALDI-HRMS and ion mobility-aligned MS/MS workflows for lipidomic analysis of zebrafish (*Danio rerio*) exposed to various xenobiotics. Zebrafish are a powerful model organism for toxicology studies due to their physiological similarities to humans. Various MALDI-MS matrices, including 2,5-DHB, ?-CHCA, were initially screened at different matrix concentrations to optimize lipid profiling. Analysis was performed on a MALDI-QTOF-MS timsTOF flex instrument (Bruker, Germany) with both TIMS on and TIMS off modes, and in both positive and negative ionization modes. MALDI-TIMS-HRMS analysis unveiled the lipidomic profile of zebrafish after xenobiotic exposure. The TIMS dimension enhanced the MALDI identification workflow. The additional CCS value information of the detected lipids, combined with the TIMS-aligned MS/MS data, greatly facilitated lipid annotation and enhanced the identification confidence. This approach highlights the potential of MALDI-TIMS-HRMS for advancing small molecule analysis in toxicological studies.

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3.12 Passive Sampling: Monitoring of Environmental Contaminants Fluxes Across Spatial and Temporal Scales

3.12.T-01 Furthering the Capabilities of Diffusive Gradient Passive Sampling for Per- and Polyfluoroalkyl Substances

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Per- and polyfluoroalkyl substances (PFAS) are chemical pollutants of growing concern for stakeholders including regulators, water managers, and consumers of contaminated seafood or drinking water. Due to their ubiquity, persistence in the environment, and potential for toxicity at low environmental concentrations, it is necessary to have convenient and reliable methods to measure PFAS in natural waters a challenge still being addressed by the scientific community. Passive sampling methods (in situ pre-concentration of PFAS) may be suitable for many monitoring situations. One passive sampling design successfully employed for historic contaminants (e.g., methylmercury) is the diffusive gradient in thin film sampler (DGT), but the design must also be validated for PFAS. Here, we iterate on previous PFAS-DGT studies by introducing a re-designed diffusive gradient sampler for PFAS in water and show that it reliably

measures 25 PFAS in water consistent with diffusion theory. Diffusion through the agarose hydrogel construction material was measured in the laboratory, and the sampler was evaluated in a combination of laboratory and field deployments. Diffusion and whole-sampler uptake rates consistently agreed with model predictions within $\pm 50\%$ relative percent difference, including when tested at cold temperature (5°C). Because of its accuracy and easy applicability to any PFAS compound, we suggest a model-based framework for predicting DGT sampling rate. In field and laboratory deployments, DGT samplers measured PFAS concentrations within $\pm 28\%$ of grab sample results on average in each case; better performance than co-deployed polyethylene tube passive samplers. In sum, we expand the experimentally-validated PFAS analyte list from 16 compounds (combined previous studies) to 25 PFAS, introduce a modeling framework to predict DGT uptake of even untested PFAS, and demonstrate the DGT sampler's precise and accurate performance in two field deployment campaigns (one requiring significant temperature adjustment). Based on the evidence in this study, the DGT passive sampler is a promising tool for consistently and accurately passive sampling PFAS in natural waters.

3.12.T-02 Application of Chemical Activity in Environmental Risk Assessment Using a Rapid Equilibrating Sampler: A Case Study on Perfluorooctane Sulfonate (PFOS)

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Current approaches for evaluating risks of contaminant exposure to human health and ecological receptors rely on the comparison of exposure and toxicological effect or no-effect concentrations. A key limitation of this approach is the inherent difficulty of comparing substances across different environmental media. Additionally, these methods are often restricted to one-to-one comparisons between exposure and effects concentrations in a single medium such as water or sediment. Consequently, much available information for risk assessments is not used. In this study, we explore the application of a chemical activity-based approach to facilitate the comparison of concentrations of PFAS among different environmental media and enhance the risk assessment process. Chemical activity is a thermodynamic quantity that describes the effective concentration for a substance in a sample, accounting for factors such as chemical's interaction with the media, temperature, and pressure. As such, chemical activity provides a way to better describe chemical partitioning, bioaccumulation, and toxic modes of action. It also enables the direct comparison of concentrations of a substance in different media. This study focuses on the application of activity on the behaviour and toxicity of perfluorooctane sulfonate (PFOS). A rapid-equilibrating thin-film passive sampler was developed and calibrated with a wide range of concentrations of PFOS in water and buffered saline. The thin-film sampler was subsequently used as a proxy to estimate the chemical activity of PFOS in samples. Results show a distinct relationship between the concentration measured in the thin-film and the activity of PFOS in the incubation solution at environmentally relevant concentrations. The thin-film sampler also effectively estimates the concentration, sorptive capacity, as well as partition coefficients for PFOS in serum albumin. Overall, we illustrate the measurement of chemical activity through rapid equilibration and demonstrate its potential as an integrative tool for supporting more comprehensive and effective environmental risk assessments.

3.12.T-03 Fluxes of Bisphenols and Phthalates on Trap Sediments from a Tropical Reef System of the Southern Gulf of Mexico

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The Veracruz Reef System is a natural protected area located off the coast of Veracruz in the southern Gulf of Mexico. It has been declared a Ramsar site and biosphere reserve with great biological, tourist, and economic importance. Various pollutants are present due to anthropic activity, river discharges, and marine and coastal currents. Bisphenols and phthalate acid esters or phthalates are emerging contaminants associated with plastic pollution since they are used in their manufacture. They are easily separated from these synthetic materials, making their presence in sediments feasible. In this work, sediment parameters such as organic matter and carbonates were quantified such as bisphenols and phthalates by gas chromatography coupled to a triple quadrupole mass spectrum after organic extraction and derivatization in trap sediments to determine the flux of these pollutants to the Veracruz Reef System. The high values of

organic matter and carbonates were 1.8 % and 34%, respectively, mainly sand and silt. The fluxes were calculated by the product of the sediment flux by the concentration of each pollutant and were 54.95 $\mu\text{g m}^{-2} \text{ days}^{-1}$ for the sum of bisphenols and 1588.75 $\mu\text{g m}^{-2} \text{ days}^{-1}$ for the sum of phthalates. The statistical analyses showed a positive correlation between organic matter and phthalates but not with bisphenols. In the sites with more significant anthropic influence, bisphenols' values were relatively higher, while the most protected sites registered higher values for phthalates. However, their presence in a natural environment indicates the alteration caused by anthropic activities. This work represents the first record of emerging contaminants in a Mexican reef ecosystem subject to enormous anthropogenic pressures. This study's contribution is the generation of the baseline on these two important groups of plasticizers in a Mexican region of great marine importance. This supports the need to establish monitoring focused mainly on the phthalates that are not commonly studied in coastal and marine ecosystems.

3.12.T-04 Chasing Equilibrium Partitioning with Water Using Silicone Chemometers

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Although partitioning between passive samplers and sediment or tissue in aquatic organisms may occur relatively quickly over several hours to days or weeks, approaching equilibrium partitioning with water presents a major challenge for highly hydrophobic organic compounds (HOCs), bringing equilibrium out of reach even after a few months. Our gold standard is to achieve equilibrium partitioning with water in a reasonable time frame to enable direct comparison of the concentrations in chemometers (passive equilibrium samplers for multimedia environments) equilibrated with other compartments. In this work, we focus on a small and shallow lake ecosystem with stable conditions for exploring a new approach aimed to reach equilibrium partitioning between silicone chemometers and water in a reasonable time frame. We used silicone-coated jars in situ (coated with Dowsil DC-2577 Low VOC, Dow Chemical Company, USA) for equilibration with water, with coating thicknesses of 0.6, 1.0, 2.0 and 3.5 μm ($n = 2$ each). In order to challenge the establishment of equilibrium, a novel device with three pumps was used to pump water out of the jars on site, while they were submerged in the water (94 h). Targeted analytes were hexachlorobenzene (HCB), pyrene, phenanthrene, dichlorodiphenyldichloroethylene (DDE), PCB44, PCB52, PCB101, PCB153 PCB138 and PCB118.

We used two approaches for calculating the equilibrium concentration in the chemometers exposed to water, and we modeled the uptake of the studied chemicals into the μm -thin coatings of the chemometers under the scenario of different water boundary layer thicknesses (?). Using coated jars with different thicknesses for each sample to assess the equilibration status, we could confirm the attainment of equilibrium for pyrene (log KOW 4.93), phenanthrene (log KOW 4.35) and HCB (log KOW 5.86). Furthermore, in the case of DDE and the PCBs, equilibrium was attained in the two thinner thicknesses. The results of the modeling indicated that if we consider a turbulent system ($\delta = 10 \mu\text{m}$) and an equilibration time of ~4 d, the thinner chemometers will be close to 95% of equilibrium for the PCBs, except for PCB138, agreeing with the results obtained. This exploratory work opens up for passive equilibrium sampling in water using silicone-coated jars. Equilibrium for compounds with log KOW up to 6 has been achieved with water in a few days using silicone chemometers, extending their applicability to a new environmental compartment.

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3.12.P-Th196 Passive Sampling and Non-Targeted Screening for Emerging Contaminants in Multi-Basin: Prioritization, Identification and Quantification

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Emerging contaminants (ECs), encompassing a diverse range of pharmaceuticals, pesticides and industrial

chemicals, pose a growing challenge to water quality and ecosystem health. Their chemical diversity and patterns of use often render traditional grab sampling methods inadequate for capturing the dynamic and fluctuating nature of pollutants in river systems, along with the restricted number of detectable target substances, resulting in an incomplete assessment of water pollution. To overcome these limitations, based on the energy-saving, environmentally friendly and reusable properties of passive sampling methods, combining them with non-targeted screening techniques would be an effective solution. 3D-printed portable passive sampling devices (PSDs) equipped with HLB disks were deployed at 19 river sites (different environment and pollution sources) from Yorkshire, UK, over seven days across different seasons. Meanwhile, composite river samples were collected in the same period using automatic samplers for comparison. A total of 38,829 features were extracted from passive samplers deployed in 19 river sites across Yorkshire, UK. Robust validation has successfully extracted 98% of spiked substances and a true positive rate (TPR) of 0.71 and accuracy of 0.99 for matches with MassBank. A total of 220 features were matched with the MassBank online spectral library and 2,503 were annotated by SIRIUS CSI:FingerID. 69 features had high-confidence library match (cosine similarity > 0.9; explained peaks > 2), resulting in assigned confidence of Level 2a. A further 91 features had fair library match (cosine similarity > 0.7) and were assigned Level 3c. Of the features matched confidently with MassBank, 12% have reported pharmaceutical applications, and a further 7% have drug and other medication information. 4% are food-related substances, and 3% have agrochemical uses. 24% of identified substances do not have any associated information accessible via PubChem. Identification and classification results from SIRIUS are in preparation, and analysis of feature abundance with respect to geographical location is under review. Our preliminary results indicate the viability of an integrated assessment approach for watershed health utilizing a combination of passive sampling and non-targeted screening techniques.

3.12.P-Th204 New Mathematical Tools to Model Accumulation in Diffusion Gradients in Thin-films (DGT) Passive Samplers Deployed in Soils and Sed

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The passive sampler Diffusion Gradients in Thin-films (DGT) is a very popular technique for dynamic speciation. DGT can be applied to natural waters, soil or sediments for a wide range of analytes, including cationic/anionic inorganic species, organic contaminants, etc. DGT devices comprise a holder, a binding/resin gel disc, a diffusive gel layer and a filter. The mass of analyte accumulated in the binding disc during the deployment time is quantified, from which an operational concentration c_{DGT} is computed. In soils, the resupply parameter R is obtained by dividing c_{DGT} by the total analyte concentration in the porewater. Previous modelling tools such as the one-dimensional DGT-induced Fluxes in Soils and Sediments code (1D-DIFS), fail to consider partially labile dissolved complexes or adsorption saturation at the soil sites. Our new open-source code SSOCO (Sediments and SOils with COMplexation) solves the diffusion-reaction equations including effects such as complexation of the target analyte (M) with a generic dissolved ligand, specific diffusion coefficients and porosities in gel and soil phases, and reaction rate constants for complexation and soil adsorption. Notably, SSOCO is not constrained by the assumption of excess soil adsorption sites. Also, we have analytically solved (using Laplace transforms) the particular case of a single species M diffusing through the gel and soil phases. With a suitable definition of an effective porosity and average diffusion coefficients, this analytical solution can be used in the limit of fast complexation and adsorption kinetics. The presentation will discuss the impact of several parameters (kinetic rate constants of complexation and adsorption, soil porosity, diffusive gel thickness, etc.). We also suggest a practical strategy, supported by numerical simulation, that involves using variable gel thicknesses to obtain the R peak within the typical measurement window. These mathematical tools could, in principle, be adapted to other passive samplers that rely on the reaction-diffusion of the analyte.

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3.12.P-Th205 Mobility, Bioavailability and Toxicity of Sediment Contaminants

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The MOBILITY project investigates the risks posed by contaminated sediments, focusing on the release, bioavailability, and toxicity of hydrophobic organic contaminants (HOCs) like polycyclic aromatic hydrocarbons (PAHs). These sediments act as sinks for persistent pollutants, which can be released into the water column through sediment resuspension, increasing their bioavailability and ecological risks. The project aimed to: (i) identify sediment properties affecting contaminant release, (ii) link dissolved and bioaccumulated HOC concentrations to biological effects, and (iii) demonstrate the utility of passive samplers for monitoring and risk assessment.

Experimental studies used artificial sediments spiked with four PAHs, assessing the impact of turbidity, hydrophobicity, and sediment grain size (PSD) on contaminant release and uptake by algae (*Ceramium tenuicorne*). Passive samplers quantified freely dissolved PAHs, enabling comparisons of bioavailability across experimental compartments using chemical activity as a common metric. Biological effects were assessed via photosynthesis inhibition.

Results revealed that turbidity significantly influenced PAH release, with high turbidity enhancing desorption of hydrophobic PAHs (e.g., fluoranthene), while low turbidity favored diffusion of less hydrophobic PAHs (e.g., acenaphthene). Sediment grain size also played a role, as finer sediments produced higher turbidity but lower dissolved organic carbon release, while coarser sediments facilitated PAH diffusion through organic matter. PAH uptake by algae correlated with passive sampler measurements, confirming their utility for bioavailability assessment. Algae exposure resulted in photosynthesis inhibition, highlighting PAH toxicity.

The project demonstrated the value of passive samplers for quantifying freely dissolved contaminant concentrations, which better reflect bioavailability and toxicity than traditional sediment analyses. Recommendations include incorporating passive samplers into regulatory frameworks, accounting for site-specific sediment properties, and standardizing passive sampler protocols to improve risk assessments and monitoring practices.

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3.12.P Passive Sampling: Monitoring of Environmental Contaminants Fluxes Across Spatial and Temporal Scales

3.12.P-Th193 AQUA-GAPS/MONET-Derived Concentrations and Trends of PFAS Across Global Waters

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Per- and polyfluoroalkyl substances (PFAS) are a large group of compounds that have been used in countless industrial, commercial and manufacturing processes and products for decades. The use and waste disposal of PFASs have resulted in widespread environmental contamination. Due to the high stability and mobility PFASs have been detected in all environmental compartments.

Despite restrictions on the use of certain PFAS, the legacy use of consumer products containing PFAS, their environmental persistence, and diffuse sources are likely to contribute to ongoing PFAS contamination of surface waters for years to come, highlighting the need for ongoing environmental monitoring for human and environmental risk assessment. Under the Stockholm Convention Global Monitoring Programme, water has been identified as a core matrix for monitoring PFAS, based on the evidence that water is the main transport medium for these chemicals in the environment. Moreover, the open ocean water column has been suggested to be the final sink of PFAS.

Passive sampling is emerging as an important tool for the monitoring and identification of PFAS in waters as they provide in situ concentration of samples, in some cases can increase sensitivity and provide a more representative image of PFAS contamination in comparison to grab sampling due to time-integrative

character of sampling.

In the present study, we present results on PFAS in surface waters from across the world, monitored within the AQUA-GAPS/MONET network between 2020-2024. A microporous polyethylene tube (MPT) passive sampler was applied in the campaign and a centralized sample analysis that allows for direct comparison of data while minimizing measurement uncertainty. The MPT sampler has been successfully calibrated and validated in surface water for a broad range of PFAS with long-term deployment capability and sensitivity.

The main objectives of the current study were to (i) deploy passive samplers for PFAS at a wide range of lake, estuarine, coastal, and oceanic sites across the world; (ii) determine background concentrations of PFAS across the waters of the world; (iii) assess the relation of dissolved PFAS concentrations with latitude, temperature, and population density; (iv) investigate the PFAS patterns at the sampling sites using suspect screening and non-targeted analysis methods and (v) use the information gained from the first set of deployments to optimize site distribution for long-term monitoring efforts.

3.12.P-Th194 Long-Term Monitoring of Hazardous Substances in Czech River Ecosystems

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Environmental monitoring of hazardous substances in aquatic ecosystems is essential for understanding pollution patterns and protecting environmental and human health. Persistent organic pollutants and heavy metals pose significant risks due to their ability to bioaccumulate in organisms, biomagnify through food chains and often act as carcinogens or endocrine disruptors. This study aims to evaluate temporal trends of selected contaminants across various environmental matrices in major Czech rivers over the period 2012 to 2023. The monitoring program includes annual sampling of biota (biofilm, fish, benthic organisms), abiotic matrices (sediments, suspended particulate matter) and passive samplers from 43 river sites. Passive samplers provide complementary data on dissolved contaminant concentrations, offering valuable information about pollutant bioavailability that cannot be obtained from solid matrix sampling. Results reveal distinct distribution patterns among different matrices. For example benthic invertebrates show highest benzo[a]pyrene accumulation compared to fish, while mercury concentrations are highest in adult fish, regularly exceeding environmental quality standards. Systematic monitoring of more matrices is required for a comprehensive assessment of aquatic ecosystem contamination due to uneven pollutant distribution. However, despite the extensive number of monitored parameters, environmental quality standards are established for only a limited number of substances, which remains a significant challenge for result interpretation.

3.12.P-Th195 Assessing the Spatial Distribution and Ecological Risks of Freely Dissolved PAHs in Sediments Using the Ex-Situ Method in Highly Industrialized Bay of South Korea

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Organic carbon, including black carbon or soot, can strongly bind hydrophobic organic contaminants (HOCs), thereby reducing their bioavailability. As a result, predicting contaminant concentrations or ecotoxicity in organisms based on total concentrations (C_{total}) in sediments may lead to overestimations. The bioavailable concentration of contaminants in sediments is represented by the freely dissolved concentration (C_{free}) in sediment porewater, which is considered suitable for assessing ecological toxicity. Previous studies have employed the in-situ method using passive samplers deployed directly in the field to measure C_{free} in porewater. However, the in-situ method often requires extended exposure times and, in some cases, the involvement of specialized personnel, such as scuba divers. In contrast, the ex-situ method offers the advantage of shorter exposure durations and lower costs for C_{free} measurement. In this study, the ex-situ method was used to measure the C_{free} of 16 priority PAHs in sediments from 33 sites in Busan Bay, South Korea, an area of concern for PAHs contamination from industrial activities. The concentrations of 15 PAH (excluding naphthalene) ranged from 6 to 67 ng/L. Higher concentrations (36–67 ng/L) were observed at sites with high shipping activities, including ship repairs. Across all sampling sites, acenaphthylene exhibited the highest relative contribution, exhibiting 3–4 benzene rings were the most predominant PAHs. To assess the ecological risk to benthic organisms, interstitial water toxicity units (IWTUs) were calculated. The IWTU values ranged from 0.01 to 0.12, remaining below the threshold value of 1, suggesting no anticipated ecotoxicological risk to benthic organisms. Despite the low IWTU values, continuous environmental monitoring of freely dissolved PAHs in sediment porewater using the ex-situ method is necessary in pollution-prone areas with intensive industrial activities, such as Busan Bay.

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3.12.P-Th196 Passive Sampling and Non-Targeted Screening for Emerging Contaminants in Multi-Basin: Prioritization, Identification and Quantification

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Emerging contaminants (ECs), encompassing a diverse range of pharmaceuticals, pesticides and industrial chemicals, pose a growing challenge to water quality and ecosystem health. Their chemical diversity and patterns of use often render traditional grab sampling methods inadequate for capturing the dynamic and fluctuating nature of pollutants in river systems, along with the restricted number of detectable target substances, resulting in an incomplete assessment of water pollution. To overcome these limitations, based on the energy-saving, environmentally friendly and reusable properties of passive sampling methods, combining them with non-targeted screening techniques would be an effective solution. 3D-printed portable passive sampling devices (PSDs) equipped with HLB disks were deployed at 19 river sites (different environment and pollution sources) from Yorkshire, UK, over seven days across different seasons. Meanwhile, composite river samples were collected in the same period using automatic samplers for comparison. A total of 38,829 features were extracted from passive samplers deployed in 19 river sites across Yorkshire, UK. Robust validation has successfully extracted 98% of spiked substances and a true positive rate (TPR) of 0.71 and accuracy of 0.99 for matches with MassBank. A total of 220 features were matched with the MassBank online spectral library and 2,503 were annotated by SIRIUS CSI:FingerID. 69 features had high-confidence library match (cosine similarity > 0.9; explained peaks > 2), resulting in assigned confidence of Level 2a. A further 91 features had fair library match (cosine similarity > 0.7) and were assigned Level 3c. Of the features matched confidently with MassBank, 12% have reported pharmaceutical applications, and a further 7% have drug and other medication information. 4% are food-related substances, and 3% have agrochemical uses. 24% of identified substances do not have any associated information accessible via PubChem. Identification and classification results from SIRIUS are in preparation, and analysis of feature abundance with respect to geographical location is under review. Our preliminary results indicate the viability of an integrated assessment approach for watershed health utilizing a combination of passive sampling and non-targeted screening techniques.

3.12.P-Th197 Application of Passive Sampling to Identify Groundwater Pollutants from Different Sources in Irrigated Areas

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Various micropollutants may contaminate groundwater. One of the sources of these contaminants is agricultural practice, where a variety of pesticides is used for plant protection. Another source of contamination can be river water that is often used for irrigation. River contamination depends on the sources of the compounds, that can be, for example, discharged wastewater, surface water runoff from agricultural or urban land, etc. In addition to conventional groundwater sampling, passive samplers for hydrophilic compounds were used in order to characterize a pollution pattern that may be influenced also by irrigation.

In total 11 boreholes in or close by irrigated areas in different parts of the Czech Republic were selected for sampling. AttractSPE® POCIS HLB for Groundwater (Affinisep, France) passive samplers were used in the study. One set of samples was collected prior and the second one at the end of irrigation season in 2024. Samples were analyzed using LC-MS/MS (targeted analysis) a LC-HRMS (suspect screening). Several pollution patterns were found even within the same irrigation area, where either agriculture pollutants (pesticides) or municipal pollutants (pharmaceuticals) or industrial pollutants (benzotriazoles etc.) prevail. The most frequently found contaminants were metabolites of chloroacetanilide and triazine herbicides as agriculture pollutants, caffeine, carbamazepine and sulfamethazine as municipal pollutants

and 5-4-methyl 1H-benzotriazole as an industrial pollutant. Additionally we found several new groundwater pollutants such as 2-naphthalenesulfonic acid, triethyl citrate, 4-acetamidopyrene and 1,3-diphenylguanidine (municipal and industrial pollutants) that may be also relevant for the other parts of the Czechia territory.

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3.12.P-Th198 Passive Sampling as Seawater Monitoring Procedure for Environmental Control at Various Marine Locations Throughout Italy

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Concerning the ecological effects of chemical pollutants in aquatic systems, it is crucial assessing the condition of the marine ecosystem today, as it affects the economic and social lives of millions of people around the world. The significant deficiencies in monitoring the presence of contaminants of various chemical groups lead to international challenges in findings of increasingly innovative monitoring techniques, associated with their ecological and ecotoxicological impact measurements.

In the framework of the RETURN project, our group is focused on optimizing sampling campaigns for monitoring traditional and emerging contaminants and the assessing of their ecotoxicological effects and ecological risk.

Passive sampling (PS) methods are distinguished by their ease of maintenance and straightforward design. It is possible to identify pollution at levels below ng/L, as they measure the time-weighted average concentrations of both organic and inorganic substances in a range of matrices, including water.

Monitoring seawaters at several locations around Italy, including Genoa Harbour, Panarea, Vulcano, Ischia, Trieste, and Grado-Marano lagoon, is the aim of our research, together detecting any ecological risks associated with chemical pollutants under climate change scenarios.

For each location, from two to six sites were chosen for the positioning of PS, as well as for sediments and water samplings. Using a multiparametric probe, physical measurements specifically, temperature, conductivity, pH, and oxygen will be integrated.

For 21 days, the Semi-Permeable Membrane Device (SPMD) and the Polar Organic Chemical Integrative Sampler (POCIS) will be used to sample organic chemicals with lipophilic and hydrophilic substances, respectively. Mercury and metals will be sampled using Diffusive Gradients in Thin Films (DGT) for seven days.

Aliivibrio fischeri, *Artemia salina*, and *Dunaliella tertiolecta* are the organisms that will be utilised in a battery of three bioassays on polar and non-polar passive sampler extracts to find ecotoxicological implications at the investigated sites.

According to preliminary findings from the initial experiment, which was conducted in Genoa Harbour from December 2023 to January 2024, passive sampling is the simplest method to quantify both organic and inorganic pollutants. Therefore, from an analytical, practical, and economical perspective, these initial data serve as a first validation of the effectiveness of passive samplers.

3.12.P-Th199 Development of Alabaster-Cement Composite Disks for DGT

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Passive sampling techniques are valuable for estimating time-weighted average concentrations (TWAc) of various chemicals in water over both short and extended periods. However, estimating TWAc requires in-situ sampling rates that depend on variables such as water temperature, flow velocity over the absorbent, and other environmental conditions. Recently, alabaster disks developed by Glanzmann et al. have been proposed as an alternative to performance reference compounds (PRCs), which can be challenging to select for specific target compounds. Alabaster disks offer a simple and reliable means of estimating in-situ sampling rates, as their weight loss can be measured precisely with a balance. However, their use in long-term field experiments is limited by their rapid dissolution within a few days.

In this study, we developed alabaster-cement composite (ACC) plates to extend the use of Diffusive Gradients in Thin-films (DGT®) passive samplers for monitoring over several weeks. In preliminary

experiments, we prepared small plates from mixtures of alabaster and cement in varying concentrations, suitable for DGT® passive samplers. We then deployed DGT® samplers with ACC plates in coastal waters near Okinawa to determine in-situ weight-loss rates. Results indicated that an alabaster concentration of 15–17% was optimal for durability and sampling performance.

Further laboratory calibration tests were conducted to assess the relationship between the weight-loss rates of ACC plates and water velocity. Ongoing calibration tests are now examining sampling rates of UV-absorbing chemicals in different water flow conditions using DGT® samplers with these composite plates. This study demonstrates the potential of ACC plates for DGT® samplers to reliably estimate TWAc of UV-absorbing compounds in coastal waters and beach environments over extended periods.

3.12.P-Th200 EXPOSO-METER: Characterizing of Lifelong Exposure to Environmental Mixtures of Pollutants (Exposome) of High-Trophic Arctic Marine Mammals

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EXPOSO-METER aims to assess environmental exposure to chemicals through practical, economical, and easy-to-use silicone-based passive equilibrium sampling. These allow identification, characterization, and direct comparison of mixtures of environmental pollutants across multiple organisms, species, and matrices. The chemometers provide advanced chemical profiling that integrates the lifelong exposome of environmental organisms to mixtures of pollutants. By defining them as a common reference phase, the chemical concentrations in the chemometers at equilibrium can be directly compared between organisms, circumventing normalizations. In this work, silicone chemometers have been used to analyze regulated and emerging hydrophobic organic pollutants present in 84 lipid-rich tissues from Arctic marine mammals collected in the surroundings of Tasilaq and Kulusuk, at the southeast coast of Greenland. Samples of pilot whales, white-sided dolphins, white-beaked dolphins, narwhals, orcas and polar bears were analyzed. Around 100 compounds were quantified by gas chromatography coupled to high-resolution mass spectrometry (HRMS), including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), pyrethroids (PYRs), chlorinated hydrocarbons (CHCs), organochlorine pesticides (OCPs), musks, UV filters and antioxidants. The results indicate that PCBs and OCPs were the predominant compound groups across all species when considering the fraction of each group of compounds in the total mixture. There was substantial variation between species, with, e.g., PCBs ranging from 35% in white-beaked dolphins to 68% in polar bears. Other groups of chemicals, such as musks, PAHs, and PYRs, showed largely differing percentages across species, ranging over two orders of magnitude. The results also indicate interspecies differences in contaminant exposure, showing a clustering by species when a principal components analysis is applied, but no clear intraspecies differences, evaluated as sex- or age-related patterns. To further extend the list of explored pollutants, especially among the legacy compounds, further additional processing based on high-resolution filtering (HRF) score and NIST library search is currently being applied in Compound Discoverer 3.3. Within this project, chemometers will help to capture and understand the exposome in Arctic top predators regarding mixtures of environmental pollutants.

Disclaimer/Disclosure: The authors acknowledge funding of first-time appointments of excellent women scientists (EXPOSO-METER, W2/W3-126). Some of the analytical instruments used in this study are part of the major infrastructure initiative CITEPro (Chemicals in the Environment Profiler) funded by the Helmholtz Association.

3.12.P-Th201 Pioneering Pollutant Detection: A Novel Passive Sampling Method for Analysing Persistent Organic Pollutants in Antarctic Sea Ice

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The project recording the baseline before the change: First steps towards an integrated chemical and

biological pollution and effects assessment off Dronning Maud Land aims to investigate the concentrations of bioavailable anthropogenic pollutants and their effects on Antarctic ecosystems. By examining pollutants like polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides, this study underscores the significance of noninvasive sampling methodologies that maintain the pristine balance of these ecosystems.

Materials and Methods: The Antarctic approach involves transporting non-living biota samples, snow, ice, and water from the Atka Bay ice shelf to Neumayer Station III, where sample preparation is performed without altering the environment. This noninvasive capability is crucial for operating within the constraints of the Antarctic's pristine ecosystems. Methodological development for the project focuses on comparing the efficacy of Stir Bar Sorptive Extraction (SBSE) with solid-phase extraction (SPE). The evaluation of analytical parameters emphasizes sustainability, reproducibility, stability, and sensitivity, aligning scientific goals with environmental stewardship in the Antarctic context. In this project stir bars coated with polydimethylsiloxane (PDMS) are used for the determination of POPs in snow, ice and water samples. The SBSE method is coupled to thermal desorption (TD)-GC-MS to determine ultratrace level (sub-ng/L to ng/L) of POPs in Antarctic aqueous samples. A stir bar (Twister; GERSTEL GmbH) coated with 24 μ l of PDMS is used for SBSE.

Results and Discussion: Preliminary experiments focused on optimizing SBSE parameters, revealing high sensitivity and reproducibility. Main experiments further validated the SBSE-GC/MS method's robustness under challenging conditions. Calibration with concentration standards ensured precise quantification, demonstrating the method's effectiveness in detecting and quantifying targeted pollutants at low concentrations.

Conclusions: This research established a robust SBSE-GC/MS protocol with high sensitivity and precision. The method proved repeatable and accurate, withstanding challenging Antarctic conditions, thus offering a reliable tool for understanding the distribution and impacts of anthropogenic pollutants in the region. These advancements support ongoing global conservation efforts while maintaining the region's environmental integrity.

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3.12.P-Th202 Laboratory Performance Study on Chemical Analysis of Hydrophobic and Hydrophilic Compounds in Two Aquatic Passive Samplers

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Quality assurance and quality control of chemical analyses are fundamental for analyses in laboratories, but also for passive sampling-based environmental assessments. The present study reports on a proficiency testing (PT) program, organised by WEPAL-QUASIMEME, for the chemical analysis of hydrophobic organic compounds (HOC) in silicone passive samplers and polar organic compounds (POC) in polar organic chemical integrative samplers (POCIS). A PT development exercise for passive sampling of HOC was initiated in 2014. Since then, six and two rounds of the exercise have been organised for the analysis of HOC and POC, respectively. The design of the study was common passive samplers were deployed for several weeks in surface water or treated wastewater, and homogenised sampler aliquots were distributed to participants for chemical analysis by methods routinely established in their laboratories. The number of participants in all rounds varied from 15 and 25 for HOCs and from 17 to 18 for POCs. Analysed HOC groups included polycyclic aromatic hydrocarbons, polychlorinated biphenyls, organochlorinated pesticides and polybrominated diphenyl ethers, whereas POC included pharmaceuticals, currently used pesticides and PFAS. The primary focus of the study was on the variability of the chemical analysis of passive samplers rather than on variability associated with sample preparation, deployment, and modelling, but the consequences for the uncertainty in estimates of aqueous concentrations are discussed. The median between-laboratory coefficients of variation (CV) of HOC analysis in silicone sampler over the previous five rounds of the PT was 32%, but the previous two rounds of POC analysis in POCIS resulted in a much higher CV of 50%. The variability for POC is high compared with the requirements of

environmental monitoring programs. Repeated exercise allows laboratories to improve their performance from lessons learned in the previous rounds. The figures from previous rounds indicated especially that the elevated uncertainty of POC data reported from polar passive samplers is not only associated with the inherent variability of sampling, but also the insufficient quality of the laboratory analysis. The implications are that proficiency testing programs may give more realistic estimates of uncertainties in chemical analysis than within-laboratory quality control programs and that these high uncertainties should be taken into account in environmental assessments.

3.12.P-Th203 Determining Polyoxymethylene-Water Partition Coefficients for 67 Polycyclic Aromatic Compounds (PACs) and Applying Them to Determine the Contaminant Availability in Thermally Treated Contaminated Soil

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Due to the different physiochemical properties of polycyclic aromatic compounds (PACs), the environmental behavior and fate of these compounds are different. This is the result of some compounds being more polar, e.g., oxygenated PACs and NSO-heterocyclic PACs, which results in them being more mobile in the environment. As soil has the ability to act as both a sink and secondary source of organic contaminants like polycyclic aromatic compounds, when remedial efforts are conducted, a question that needs to be investigated is whether the remedial efforts have any impact on the contaminant availability and consequently its environmental mobility and toxicity.

Passive sampling methods such as the polyoxymethylene (POM) method facilitates the determination of the freely dissolved concentration of the organic contaminants of interest, i.e., PACs in this study. By utilizing the POM method to determine the availability of PACs prior to and after remedial efforts have been conducted, both the effectiveness of the remediation technique implemented and any changes in contaminant availability can be assessed.

Currently, POM-water partitioning coefficients have been established for 33 PACs. Therefore, in this study, the number of PACs with determined POM-water partition coefficients have been expanded from 33 to 67 compounds, encompassing native, alkylated, oxygenated, and NSO-heterocyclic PACs. These partition coefficients will be used to determine the soil porewater concentrations in soil samples taken prior to and after thermal remediation of a former gasworks site to determine whether there are any changes in the soil porewater concentrations after remedial efforts have been conducted.

3.12.P-Th204 New Mathematical Tools to Model Accumulation in Diffusion Gradients in Thin-Films (DGT) Passive Samplers Deployed in Soils and Sed

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The passive sampler Diffusion Gradients in Thin-films (DGT) is a very popular technique for dynamic speciation. DGT can be applied to natural waters, soil or sediments for a wide range of analytes, including cationic/anionic inorganic species, organic contaminants, etc. DGT devices comprise a holder, a binding/resin gel disc, a diffusive gel layer and a filter. The mass of analyte accumulated in the binding disc during the deployment time is quantified, from which an operational concentration c_{DGT} is computed. In soils, the resupply parameter R is obtained by dividing c_{DGT} by the total analyte concentration in the porewater. Previous modelling tools such as the one-dimensional DGT-induced Fluxes in Soils and Sediments code (1D-DIFS), fail to consider partially labile dissolved complexes or adsorption saturation at the soil sites. Our new open-source code SSOCO (Sediments and SOils with COmplexation) solves the diffusion-reaction equations including effects such as complexation of the target analyte (M) with a generic dissolved ligand, specific diffusion coefficients and porosities in gel and soil phases, and reaction rate constants for complexation and soil adsorption. Notably, SSOCO is not constrained by the assumption of excess soil adsorption sites. Also, we have analytically solved (using Laplace transforms) the particular case of a single species M diffusing through the gel and soil phases. With a suitable definition of an effective porosity and average diffusion coefficients, this analytical solution can be used in the limit of fast complexation and adsorption kinetics. The presentation will discuss the impact of several parameters (kinetic rate constants of complexation and adsorption, soil

porosity, diffusive gel thickness, etc.). We also suggest a practical strategy, supported by numerical simulation, that involves using variable gel thicknesses to obtain the R peak within the typical measurement window. These mathematical tools could, in principle, be adapted to other passive samplers that rely on the reaction-diffusion of the analyte.

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3.12.P-Th205 Mobility, Bioavailability and Toxicity of Sediment Contaminants

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The MOBILITY project investigates the risks posed by contaminated sediments, focusing on the release, bioavailability, and toxicity of hydrophobic organic contaminants (HOCs) like polycyclic aromatic hydrocarbons (PAHs). These sediments act as sinks for persistent pollutants, which can be released into the water column through sediment resuspension, increasing their bioavailability and ecological risks. The project aimed to: (i) identify sediment properties affecting contaminant release, (ii) link dissolved and bioaccumulated HOC concentrations to biological effects, and (iii) demonstrate the utility of passive samplers for monitoring and risk assessment.

Experimental studies used artificial sediments spiked with four PAHs, assessing the impact of turbidity, hydrophobicity, and sediment grain size (PSD) on contaminant release and uptake by algae (*Ceramium tenuicorne*). Passive samplers quantified freely dissolved PAHs, enabling comparisons of bioavailability across experimental compartments using chemical activity as a common metric. Biological effects were assessed via photosynthesis inhibition.

Results revealed that turbidity significantly influenced PAH release, with high turbidity enhancing desorption of hydrophobic PAHs (e.g., fluoranthene), while low turbidity favored diffusion of less hydrophobic PAHs (e.g., acenaphthene). Sediment grain size also played a role, as finer sediments produced higher turbidity but lower dissolved organic carbon release, while coarser sediments facilitated PAH diffusion through organic matter. PAH uptake by algae correlated with passive sampler measurements, confirming their utility for bioavailability assessment. Algae exposure resulted in photosynthesis inhibition, highlighting PAH toxicity.

The project demonstrated the value of passive samplers for quantifying freely dissolved contaminant concentrations, which better reflect bioavailability and toxicity than traditional sediment analyses. Recommendations include incorporating passive samplers into regulatory frameworks, accounting for site-specific sediment properties, and standardizing passive sampler protocols to improve risk assessments and monitoring practices.

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3.12.P-Th206 Atmospheric Deposition of Airborne Microplastics in Urban Background Site in Helsinki

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Urban environments are increasingly affected by microplastic and tire wear rubber pollution, which pose ecological and human health risks. Since the production of plastics is annually increasing and their applications are broadening, there are potentially myriad types of microplastics sources. Microplastics have been shown to spread in the environment via rivers, wastewater treatment plants, stormwaters, soils and atmosphere. Even though these pathways have been recognized and explored, the results are often not comparable partly due to the unharmonized methodology. In this study, the influence of sampling height and the repeatability of sampling are investigated.

Wet and dry deposition samples were collected as three parallel samplings at a height of 4 m (60.20288, 24.96125) and 15 m (60.20342, 24.96088) from ground level in Kumpula campus of University of Helsinki (N60.20, 24.96) that represents an urban background site. The 14-day samples were collected with in-house, plastic-free samplers (sampling area 20 cm in diameter) between April 8 and May 20, 2024.

Air quality data and meteorological data including temperature, wind speed and direction, relative humidity and precipitation were continuously collected at the SMEAR III sampling station (60.20288, 24.96125). Samples were analyzed with a gas chromatography-mass spectrometer (GC-MS; Shimadzu QP2020NX) coupled with a microfurnace pyrolyzer (Py; Frontier Laboratories EGA-PY-3030D). The vertical distribution of the mass concentrations of four plastics (polyethylene, polypropylene and polystyrene) and tire rubbers (styrene butadiene and natural rubber) will be presented. In addition, the deviations between the three parallel samplings during different events (snowing, raining and dry period as well as varying pollen conditions) will be discussed.

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3.12.P-Th207 Ambient Air Contamination: European Assessment of Pesticide Residues in Ambient Air Using Passive Sampling

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The intensive and widespread use of pesticides, sometimes including misuse, has led to their detection in various environmental matrices, including ambient air. Pesticides are particularly important in agriculture for plant protection and subsequently increased yields. At the same time, agricultural practices, particularly spraying, are a significant source of pesticide residues in rural and surrounding areas. Some of these residues, e.g. organic pollutants and some current-use pesticides, persist in the air and can travel long distances. However, knowledge of the distribution and the atmospheric transport of pesticides is still incomplete. This study aims to assess the occurrence and possible levels of multiple pesticide residues in European ambient air using passive air sampling as a harmonized and emerging cost-effective method. This study was part of the monitoring campaign of the SPRINT project covering 10 European countries and primary crop types in Europe, under conventional (CONV), and organic (ORG) system. Ambient air sampling occurred during the growing season using passive samplers (TIEM, Germany), which combined polyurethane foam (PUF) and polyester (PEF) filters. For PEF filters, Glyphosate and aminomethylphosphonic acid (AMPA) were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS), with a modified QuEChERS extraction method. PUF filter extractions were analyzed through LC MS/MS and GC MS/MS. Out the 161 compounds analyzed, 75 were quantified, and 8 were not approved in the EU at the sampling date (2021). Glyphosate (100%), AMPA (75%), and pendimethalin (70%) are the most frequently quantified residues. DDE p,p', a banned pesticide, was detected in 25% of the samples. Our results revealed the occurrence of multiple pesticide residues in the ambient around both conventional and organic fields. The number of pesticides quantified per site ranged from 3 to 26 residues. Across all the Case Study Sites (CSS), the highest number of residues next to conventional fields occurred in the Netherlands (n = 26), while the highest number adjacent to organic fields occurred in Portugal (n = 26).

Results show several remarkable patterns on pesticide residue mixture profiles. Complex mixtures of pesticides are ubiquitously spread in ambient air suggesting the need for a systematic monitoring of pesticide in ambient air within agricultural and surrounding areas.

3.12.P-Th208 Passive Sampling of Persistent Organic Chemicals Under Free Tropospheric Conditions

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The free troposphere (FT) is defined as the middle and upper part of the troposphere that dynamically extends from the top of the planetary boundary layer (PBL) up to the tropopause. Compared to the PBL, the FT is characterized by lower atmospheric pressure, increased wind speed, and high solar radiation causing differences in the physicochemical behaviors of organic chemicals. High-altitude regions are in near-constant contact with the FT, and thus provide the opportunity to investigate the levels and behaviors of organic pollutants in such unique conditions. Many studies have reported persistent organic chemicals (POCs) in remote environments like the Antarctic and Tibetan Plateaus, and Himalayas that clearly emphasize long-range atmospheric transport (LRAT) and cold-condensation effect. However, the role of FT in these processes still needs to be clarified. Moreover, previous studies often focus on specific

altitudes and lack data across altitudinal gradients. Therefore, it is crucial to reach sites where FT conditions occur at different frequencies and where episodes in which air parcels from the PBL are transported to the site vary. Chacaltaya Mountain, in the range of Bolivian Andes, was selected as the study site within this work. Sixteen polyurethane foam (PUF) passive air samplers (PASs) were deployed in 8 sampling points spanning altitudes from 3600 (City Center of La Paz) to 5240 masl. Additionally, 4 PAS-PUFs equipped with automatic activation/deactivation setups were mounted at the summit (approx. 5380 masl) to continuously monitor day-long fluctuations of atmospheric POCs. The setup was designed to track the influence of FT conditions on these fluctuations by employing each unit for selected 6 hour periods in a day. Before they were deployed, all the PUF samplers were spiked with isotopically labeled compounds having a range of volatilities to determine compound-specific sampling volumes at the relevant conditions. Although the effects of the wide range of ambient temperatures and wind speeds on passive air sampling have already been reported in previous studies, atmospheric pressure has not been fully explored as a parameter that determines PAS sampling rate in this context. Thus the other aim of the present work is to investigate the possible effects of low atmospheric pressures (i.e., approx. 0,51 atm at 5240 masl) on uptake rates of gas-phase POCs by PAS-PUF samplers.

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3.12.P-Th209 Determination of NO₂, SO₂ and O₃ Levels in Ankara, Türkiye: Assessment of the Exposure of Primary School Children

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The quality of urban air, particularly in regard to the concentrations of nitrogen dioxide (NO₂), sulfur dioxide (SO₂) and ozone (O₃), presents a significant health risk, particularly for those populations considered vulnerable, including children. This study includes the passive sampling of NO₂, SO₂, and O₃ along routes frequently traversed by primary school children in a large urban area in Ankara, Türkiye. Novel passive samplers developed by Eskişehir Technical University Air Quality Research Group were deployed at 160 points on the walking paths from schools to students' homes to obtain pollutant concentrations. The primary aim was to assess the exposure of children to air pollutants during their daily commutes, given that these routes often intersect with high-traffic zones. The data collected in two sampling campaigns (winter and summer) over a two-week period were subjected to analysis in order to identify any spatial or temporal variations in pollutant concentrations. The highest concentrations of NO₂, SO₂ and O₃ obtained during the initial sampling period (winter) were 78.58, 34.59 and 99.53 µg/m³, respectively, while the corresponding sampling results for the subsequent sampling period (summer) were 73.63, 88.71 and 182.58 µg/m³. The findings provide valuable insights into the micro-environments that children are exposed to and highlight the importance of targeting pollution reduction strategies in specific urban pathways. This study contributes to the growing body of research on air quality monitoring and offers a unique perspective on children's exposure to urban air pollutants.

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3.13.A Advances in Bioaccumulation Science and Assessment

3.13.A.T-01 Irreversible Receptor Binding Causes Long-Lasting Bioaccumulation of Insecticides in Two Invertebrate Species

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Monitoring data revealed that polar compounds such as neonicotinoids substantially influence the total toxic pressure on non-target invertebrates, posing environmental concerns. Irreversible receptor binding to

the nicotinic acetylcholine receptors (nAChRs) may explain accumulation, potentially causing long-lasting toxic effects. Accordingly, an elimination-resistant fraction independent of concentration level and exposure dynamics has been observed for thiacloprid.

This study examines the toxicokinetic mechanisms of lasting bioaccumulation caused by receptor binding for four insecticides of different substance classes in the two freshwater invertebrate species *Gammarus pulex* and *Hyaella azteca*.

Amphipods were exposed to four polar insecticides (i.e., thiacloprid, acetamiprid, imidacloprid, and flupyradifurone), and uptake and elimination kinetics were investigated over 6 to 18 days for different exposure concentrations (5, 10, 25, 50 µg L⁻¹). Further, in vivo nAChR-binding assays were performed at the uptake and elimination phases to determine the contribution of the membrane protein extraction fraction to the total body burden. Concentrations in the medium and extracts were determined using an online-solid phase extraction system coupled with liquid chromatography, and high-resolution tandem mass spectrometry (online-SPE LC-HRMS/MS).

Results confirm an elimination resistant fraction for the three N-cyanoamidines and N-nitroguanidine insecticides (i.e., thiacloprid, acetamiprid and imidacloprid) independent of concentration levels, species, and compounds. The fraction associated with membrane proteins accounted for the largest amount of the total body burden at the elimination phase. In contrast, the newer butenolide insecticide flupyradifurone is fully eliminated and no membrane protein associated fraction could be detected.

Irreversible binding to the nAChRs and accordingly elimination resistance in both amphipod species may explain previously reported delayed toxicity caused by neonicotinoids. Our findings support that receptor binding as a cause for bioaccumulation should be considered in chemical risk assessment.

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3.13.A.T-02 Toxicokinetics of Per- and Polyfluoroalkyl Substances (PFAS) in Amphipods Using Online-SPE-LC-HRMS/MS

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Per- and polyfluoroalkyl substances (PFAS) represent a group of more than 9000 synthetic organic compounds detected globally as contaminants. They are used as surfactants and non-stick agents since the early 1950s. Toxicokinetic is essential for understanding the relationship between contaminants concentrations and toxic effects on organisms such as *Hyaella azteca*, which is easy to culture and considered a good alternative for animal testing with fish (i.e., OECD 305). The general objective of this work is to determine PFAS toxicokinetics in *H. azteca* and applying a state-of-the-art analytical methodology. We want to compare PFAS with different functional groups and chain lengths as well as correlate concentration in different matrices (whole-body and hemolymph). 22 PFAS were selected and preliminary exposure and toxicity experiments were performed at concentrations observed at contaminated sites (i.e., 50 µg/L) with different PFAS mixtures in triplicate over 7 days. Then, a full toxicokinetic experiment was performed over 28 days (10 days for uptake, 18 days for elimination phase). Organisms, hemolymph, medium and food samples were sampled at different time points, extracted and analysed by online-SPE-LC-HRMS/MS. The methodology was successfully applied for different biota samples with calibration curve from 0.005 to 250 µg/L ($r^2 \geq 0.99$; CV% $\leq 20\%$). Organisms presented satisfactory survival rate ($\geq 80\%$) when preliminarily exposed with PFAS classes X:2 FTS or DiPAPs at 50 µg/L. 8:2 DiPAP presented the average concentrations: $3,720 \pm 724$ µg/kg for whole-body, $5,767 \pm 2,581$ µg/kg for hemolymph, with an average concentration ratio of 0.6, and a whole-body and hemolymph BCF values equal to 74 and 115 L/kg, respectively. 10:2 FTS, with the longest chain length, had the highest average concentrations: $121,766 \pm 72,976$ µg/kg for whole-body, $28,000 \pm 11,067$ µg/kg for hemolymph, with an average concentration ratio of 4.3, and a whole-body and hemolymph BCF values equal to 2,435 (potential for bioaccumulation) and 560 L/kg, respectively. With these results and concentrations ratios, it is possible to see that PFAS concentration can change in different matrices, which may be related to their different chemical properties. In the next steps, the full toxicokinetic experiment data (28 days) will be analysed, toxicokinetic modelling will be performed and the final results will be presented.

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3.13.A.T-03 Bioaccumulation and Biomagnification of Per- and Polyfluoroalkyl Substances at Different Trophic Levels in the Food Web of a Polluted Ecosystem

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Per- and polyfluorinated substances (PFAS) are widely used anthropogenic compounds, with a multitude of industrial and consumer applications. The widespread use of PFAS together with their persistence and mobility has led to global contamination of the abiotic and biotic environment. Nevertheless, important knowledge gaps remain concerning their occurrence, bioaccumulation and biomagnification across aquatic ecosystems, while even less is known about terrestrial ecosystems. The aim of the present study was therefore to unravel the aquatic and terrestrial pathways of PFAS in a polluted ecosystem, ending up in predatory spiders as top predators. To this end, abiotic and biotic samples from the aquatic and terrestrial compartments of a PFAS polluted ecosystem were collected and screened for 44 PFAS. The food web structure and trophic relationships between the organisms were investigated using stable isotopes and a mixing model analysis. Sampled organisms were grouped according to their trophic level into primary producers (PP), primary consumers (PC) and predators (PR). The quantified concentrations revealed that a wide range of PFAS is present in the abiotic and biotic environment with total average concentrations reaching up to 11 µg/g dw in the case of aquatic PC, corresponding to 34 detected PFAS with PFOS constantly appearing among the compounds with the highest concentrations. Biota-to-soil and biota-to-sediment accumulation factors (BSAFs) confirmed the high bioaccumulation potential of PFAS, with BSAF values reaching 91,971 kg sediment/kg biota. Similarly, a high bioconcentration potential from water was observed, with bioconcentration factors (BCFs) reaching 33,087 L water/kg biota. Considerable variation was observed in the bioaccumulation between the different trophic levels, but also between the different species. Furthermore, biomagnification of PFAS through the food web appeared to depend both on the intrinsic PFAS properties, and on the trophic interactions within the examined ecosystem. Our results highlight that a wide spectrum of PFAS detected in terrestrial and aquatic environments can be taken up by organisms from different trophic levels. PFAS biomagnification is substantial and hence, food web transfer of PFAS is of concern, contributing to the environmental risks of these forever compounds.

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3.13.A.T-04 Benchmarking Approach in Bioaccumulation Assessment

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Recently updated ECHA Guidance on information requirements and chemical safety assessment (IR&CSA), Chapter R.11 provides general recommendations on how to apply a benchmarking approach as part of an overall weight of evidence in PBT/vPvB assessment. The benchmarking approach is defined as the approach where the fate or behaviour of a substance is measured relative to the fate or the behaviour of a well-described substance(s), the so-called benchmark chemical(s). Benchmarking relies on the similarity between the fate or the behaviour of the benchmark chemical and the substance of interest in the environmental system being studied. It is a comparative method, and, unlike the read-across approach, it does not require structural similarity.

In this presentation, a benchmarking approach in addressing bioaccumulation potential based on information on fish dietary exposure bioaccumulation studies will be presented with neutral organic substances as illustrative case examples. The studies have been collected for substances identified as bioaccumulative (B) or very bioaccumulative (vB) and as Substances of Very High Concern (SVHC) according to REACH Article 57 (d) or (e), with relevant parameters (e.g. physico-chemical properties of a substance, experimental design and test conditions) that may influence the outcomes of the tests. The factors which may significantly influence the outcome of dietary studies and hence should be considered in the benchmarking approach will be discussed in detail. Potential for benchmarking approaches based on

other bioaccumulation assessment scenarios than dietary fish data will also be presented.

The benchmark approach demonstrates:

- What to take into account when selecting benchmark chemicals;
- Most relevant parameters which appear to influence the outcomes of the study;
- Need for consensus building on the parameters to check for benchmarking;
- Frequent data/reporting gaps, which needs to be improved, to allow for meaningful comparison of studies.

In the future, benchmark examples for so called 'safe case' substances that have been concluded as not bioaccumulative will be included. This is expected to further facilitate the use of available dietary studies to conclude on the bioaccumulation potential of other substances. We will provide an overview of possible bottlenecks with the benchmarking approach, as well as suggestions for further development for other types of substances (e.g. ionisable and surface-active substances).

3.13.A.T-05 Assessment of the Bioaccumulation of Organic Pollutants Using Zebrafish Liver Cells and Evaluation of the Important In Vitro Parameters

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The development of alternative methods that link cellular and predictive toxicity to high-level toxicity is a key focus of current research within the framework of the 3Rs (Reduce, Replace, Refine) in animal experimentation. In the field of bioaccumulation, significant progress has been made through the development of empirical predictive regression models and in vitro-in vivo extrapolation (IVIVE) techniques using rainbow trout hepatocytes. However, substances with slow metabolism rates face limitations with 4-hour assays. Additionally, these models often rely on the octanol-water partition coefficient of compounds to evaluate absorption, the depletion of the parent compound to assess metabolism, or mass balance models (MBMs) to determine internal concentrations without accounting for the kinetics of absorption and metabolism. This approach overlooks crucial information, as biological processes occur simultaneously and interact dynamically.

This study assessed a methodology employing the zebrafish liver cell line (ZFL) to predict the bioconcentration factor (BCF). A miniaturized analytical method was utilized to measure the concentrations of the parent compounds (BDE-47 and phenanthrene) and their primary metabolites in the exposure medium and within the cells (C_{cell,exp}). The cells were cultured on glass plates and exposed for varying durations of up to 72 hours. Conversely, the bioavailable concentration (C_{free}) and the predicted internal concentration (C_{cell,MBM}) were determined using mass balance modeling. Bioconcentration factors (BCFs) were calculated by integrating these values, applying toxicokinetic modeling, and accounting for metabolism through the IVIVE model.

The results revealed discrepancies between C_{cell,exp} and C_{cell,MBM}, indicating that the most accurate BCFs, closely aligned with in vivo values, were achieved by incorporating C_{cell,exp}, C_{free}, and the uptake and biotransformation kinetics of the compounds. This study confirmed the capability of ZFL cells for BCF prediction, demonstrating a rapid and animal-free methodology. The findings provide valuable data for refining predictive models.

3.13.B Advances in Bioaccumulation Science and Assessment

3.13.B.T-01 Consensus for a Better "B"-Assessment

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Bioaccumulation assessment is an essential part in all regulatory frameworks on chemicals. In the past years, there have been significant developments to incorporate the demands of the 3Rs (reduce, replace, refine animal testing) and to extend the bioaccumulation assessment to substances other than neutral, lipophilic substances, and to air-breathing organisms, where necessary. These advancements should be considered in revised European testing and assessment guidances.

The German Environment Agency has initiated a project with the aim of integrating current scientific advancements into a comprehensive bioaccumulation assessment concept. Multiple lines of evidence are

combined, based on in silico methods, in vitro assays and tests with invertebrates in addition to existing in vivo data and results from environmental monitoring. Their consolidation by consensus approaches includes an evaluation of the applicability and uncertainties with due regard to regulatory thresholds. Several building blocks were addressed in the project, such as a consensus modelling approach for consolidated log Kow and log Koa values and recommendations for obtaining reliable and robust pKa estimates. Also, fish BCF QSAR data was generated from several available QSAR models. For 30 substances, fish BCF data was compared to results of *Hyalella azteca* bioconcentration tests (HYBIT) according to OECD TG 321, in vitro assays (OECD TG 319 A/B) for the assessment of the bioaccumulation potential of substances in aquatic species and in vitro depletion assays with rat hepatocytes/ S9 fractions to assess the bioaccumulation potential in air-breathing organisms. Where literature data was not complete, HYBIT tests and both fish and rat in vitro tests were conducted to complete the data set.

The consensus modelling improves the reliability of B-assessments based on consolidated predictions and test results, leading to reduced uncertainties.

On the basis of a dedicated workshop with the European regulatory community from the different substance regulations in March 2025, a revised proposal will be presented together with the conclusions of the workshop.

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3.13.B.T-02 Determining the Uptake and Depuration Rates of Polycyclic Aromatic Compounds in a Popular Biomonitoring Species

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Polycyclic aromatic compounds (PACs) are ubiquitous contaminants that are commonly measured in biomonitoring programs, and the bioavailability of each PAC can be determined by physical-chemical properties like the octanol-water partitioning coefficient (Kow). Though uptake and depuration rates of PACs in mussels have been previously studied, some data gaps remain in our understanding the toxicokinetics of these compounds in tissue. The goal of this study was to address these gaps by measuring the accumulation and depuration rates of a suite of PACs in mussels exposed to different oils and pre-made PAC blends. These measurements allowed us to test whether water PAC composition is predictive of tissue PAC profiles, if Kow is predictive of the uptake and depuration rates of PACs within complex mixtures, if artificially made PAC blends are reflective of the toxicokinetics of crude oil mixtures, and to determine how rapidly uptake can occur in an acute exposure scenario. Two experiments were conducted. For the first experiment, 7-day exposures to two different types of crude oil (a light and a heavy crude oil) followed by a 14-day depuration period were conducted. Mussel samples were pooled for PAC tissue analysis on days 0, 3, 7, 10, 14, and 21. In the second experiment 72h exposures were conducted with light crude oil, a PAC blend. Mussels were sampled at 0, 3, 6, 24, 48, and 72h during the exposure phase, and 10 and 17 days during the depuration phase of the study. Water samples were taken during the exposure period for PAC and biomimetic extraction-solid phase microextraction (BE-SPME) analysis for both studies. In the oil comparison study, the light crude oil had higher concentrations of the lower molecular weight PACs accumulated in the mussel tissue compared to the heavy oil. In our second experiment, we found that the PACs accumulated in the mussel tissue rapidly, with ~1000ng/g concentrations of naphthalene and 1-methylnaphthalene measured within 3 hours of exposure. In both experiments most of the lower molecular weight (and lower Kow) compounds were completely depurated by our first sampling period post exposure. The tissue concentrations remained above baseline on the last day of the trial for the higher molecular weight compounds. The data generated in this study can be used to improve our understanding of the toxicokinetics of PACs in a species that is used as both a food source for human consumption and a key part of biomonitoring programs.

3.13.B.T-03 Does Dietary Microplastic Enhance the Bioaccumulation of Environmental Contaminants and Polymer Additives?

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While there is consensus on the presence of microplastic contamination in food, this consensus does not extend to the question of how this presence influences the uptake of organic contaminants from the gastrointestinal tract. Hypotheses range from the idea that microplastic acts as carrier of sorbing

contaminants facilitating biological uptake to the opposite view that the sorptive capacity of microplastic, which is not diminished during digestion, lowers the thermodynamic driving force responsible for diffusion from gut lumen to the organism's tissues. Using silicone-based equilibrium sampling we quantified the effect of polyvinylchloride (PVC) microplastic on this thermodynamic driving force, expressed in terms of fugacity, for polychlorinated biphenyls (PCBs) and polymer additives in dietary and fecal samples of a zoo-housed polar bear. Whereas PVC microplastic at concentrations well above current observations reduced the fugacities of spiked isotopically labelled PCBs in polar bear diet and feces slightly, but significantly, leaching from those microplastic greatly elevated fugacities of the additives U-328 and octabenzene in those samples. The impact of microplastic in the diet on the biological uptake of environmental hydrophobic organic contaminant is likely to be negligible. Microplastic has the potential to be an effective vector for the dietary uptake of polymer additives.

3.13.B.T-04 Comparing the Predictive Capacity of the OECD 319B In Vitro Fish Biotransformation Assay to the In Vivo OECD 305 Study for a Hydrophobic Organic Chemical

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This presentation describes the remarkable potential and capacity of the OECD 319B Rainbow Trout liver S9 fraction in vitro assay to accurately predict not only the bioconcentration potential of organic chemicals, but also the metabolic pathway of transformation products formed in the traditional OECD 305 In vivo fish bioconcentration study, which is still the gold standard test for establishing the bioconcentration factor (BCF) of chemicals. Two high quality studies were performed on the hydrophobic organic cyclic acetal fragrance molecule Okoumal (EC No. 412-950-7), which has an experimentally determined LogKOW of 5.15. The first study, an OECD 305 in vivo fish bioconcentration study, followed the uptake and depuration of the ¹⁴C-radiolabelled test item in the tissue of Fathead Minnow (*P. promelas*), and identified the major metabolite formed in the fish tissue. The second study, an in vitro fish metabolism study using Rainbow Trout (*O. mykiss*) liver S9 fractions according to the OECD 319B test guideline was conducted with non-radiolabelled Okoumal. In the in vivo study, a steady-state BCF of 412 L/kg (wet wt.), normalised to 5% lipid content, was determined for the parent molecule, attaining a plateau within the first 7 days of exposure. The in vitro study demonstrated high metabolic turnover (90% of starting material) with a relatively rapid intrinsic clearance rate. The predicted BCF from the in vivo to in vitro extrapolation (IVIVE) model of Nichols et al. (2013) of 309 L/kg (wet wt.), in close agreement with the In vivo result. The principal metabolite, representing 60% of total C14 in fish tissue in the in vivo OECD 305 study, was identified as the Hydroxy-Glucuronide of the parent molecule, and was rapidly eliminated from the fish with a tissue half-life of between 1.0 and 1.8 days. The same structure was also identified putatively as a major Phase II biotransformation metabolite in the In vitro study, confirming the utility of the OECD 319B assay as not only an efficient and fit-for-purpose screening approach which can be employed to de-/prioritise the requirement for full in vivo testing, but is also capable of predicting similar mechanistic metabolism pathways as the in vivo study supporting the rationale that the OECD 319B should be integrated in to a systematic tiered assessment approach for evaluating bioaccumulation potential.

3.13.B.T-05 Adapting In Vitro Substrate Depletion Assays (TG 319) to Study Biotransformation of Organic Chemicals in Mallard Duck

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Avian species play a critical role in aquatic and terrestrial ecosystems by providing many ecosystem services, and historically have been significantly impacted by the bioaccumulation of organic pollutants due to their trophic positioning, diverse life traits, and complex migration patterns. Bioaccumulation is typically assessed in fish using B-metrics such as bioconcentration/bioaccumulation factors (BCF/BAF) depending on exposure route; however such methods are challenging to apply to air breathing organisms, but are often considered protective. These metrics are determined using costly in vivo testing in fish and rats, with little focus on birds outside of field studies which have inherent modelling limitations. Recently, it has been suggested that a kinetic approach that views these B-metrics as the product of competing rates of whole-body uptake and elimination may be refined by incorporating tissue-specific biotransformation

rates derived from substrate depletion assays using isolated hepatocytes or liver S9 sub-cellular fractions. The present study seeks to optimize, evaluate, and apply these assays to multiple relevant bird species using a diverse suite of benchmark compounds, by adapting the Organisation for Economic Co-operation and Development (OECD) Test Guideline 319A/B. In initial studies using mallard duck (*Anas platyrhynchos*) metabolic material, in vitro intrinsic clearance rates of pyrene and 4-n-nonylphenol were consistently determined across multiple assay conditions, with various thawing and handling protocols to test for robustness. Further research will apply an optimized assay protocol to additional relevant chemicals including anticoagulant rodenticides, polychlorinated biphenyls (PCBs), organochlorines, and emerging contaminants of wildlife concern. Such clearance data can be used flexibly within integrative frameworks to refine predictions of bioaccumulation. The goal of this study is to generate consistent data for tiered bioaccumulation assessment strategies, as well as lay the foundation for future ring trial or similar applications to inform wildlife risk assessment.

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3.13.P Advances in Bioaccumulation Science and Assessment

3.13.P-Th210 Why the Bioconcentration Factor (BCF) Fails for Superhydrophobic Chemicals

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Assessing the bioaccumulation potential of superhydrophobic chemicals is extremely challenging. Next to problems generally encountered when testing superhydrophobic chemicals in experimental systems, there are specific influencing factors in bioconcentration (BCF) tests that have apparently not been sufficiently considered in current guidelines (e.g. OECD 305). Effects such as binding of chemicals to organic carbon (TOC) in the test medium, significant growth dilution and elimination via feces, and (extreme) times till steady-state make performance, interpretation and validity of bioconcentration tests for superhydrophobic chemicals extremely challenging. These effects can lead to the counter-intuitive observation of decreasing BCF in the superhydrophobic range with increasing octanol/water partition coefficients K_{ow} , even in the absence of biotransformation.

To address this problem, the German Environment Agency (UBA) launched a project with the objectives to i) provide a precise definition of superhydrophobicity, ii) to quantify whether and to what extent the bioaccumulation potential of superhydrophobic substances has been underestimated so far, iii) to examine existing tests and models for their suitability in assessing the bioaccumulation potential of superhydrophobic compounds, iv) to provide a concept and proposals for revision of guidance documents for the future bioaccumulation assessment of superhydrophobic compounds and to v) communicate and find an agreement regarding the approaches with the expert community.

This abstract and the respective poster presentation at the start of the project strive to create awareness for the problems regulators are facing when assessing superhydrophobic compounds for bioaccumulation, elucidate first project outcomes and encourage discussions that will ultimately help achieving an improved bioaccumulation assessment in Europe.

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3.13.P-Th211 How Does Active Transport affect Toxicokinetics of Cationic Psychoactive Drugs in Aquatic Invertebrates?

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Toxicokinetics of ionic compounds are still not well understood. Recent studies on the toxicokinetics of cationic pharmaceuticals in aquatic invertebrates reported a strong influence of exposure concentration and temperature on both uptake and elimination rates of these chemicals. Both observations may be indicative for active transport mechanisms facilitated by certain membrane proteins. Such proteins include

ATP-binding cassette transporters (ABC-transporters) or solute carriers (SLCs).

The potential active transport of cationic pharmaceuticals was studied by performing uptake and elimination experiments with the freshwater amphipod *Gammarus pulex*. Different mixtures and exposure sequences of the target analyte, i.e. the antidepressant citalopram, and inhibitors of membrane transport proteins (ABC and SLC transporters), i.e. verapamil and cyclosporine A, were performed to understand the interactions of these two compound classes. Furthermore, a neutral benchmark compound (fluopyram) known to be unaffected by transporter inhibition was added to the exposure mixtures. Contaminant concentrations were determined using an online-SPE LC-HRMS/MS system. Toxicokinetic parameters were estimated using the build your own model (BYOM) platform.

The co-exposure with verapamil reduced the uptake rate of citalopram by 40% and the elimination rate by 75%, whereas cyclosporine A and other inhibitors showed no effect. Consequently, the bioconcentration factor (BCF) of citalopram was two times higher if verapamil was co-exposed during both uptake and elimination phase, and six times higher if verapamil was only co-exposed during the elimination phase. The present study provides insights into transport pathways that may be specific to cations. Furthermore, it was demonstrated that the effect of transporter inhibition could be detectable on in vivo scale for verapamil. However, it remains unclear why other inhibitors known to be efficient in in vitro experiments showed no effect. The results may help to understand why some contaminants show higher bioaccumulation potential under environmental conditions (i.e. low exposure concentrations, mixtures, matrix components such as DOM) than estimated based on highly standardised laboratory experiments.

3.13.P-Th212 Phenolic Compounds in the Freshwater Environment in South Korea: Occurrence and Tissue-specific Distribution

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In this study, we investigated the occurrence and distribution of phenolic compounds, including phenol, cresols, chlorophenols, nitrophenol, and bromophenols, in freshwater environments. We also focused on phenolic compounds in crucian carp (*Carassius auratus*) tissues, specifically the muscle, gills, brain, blood, liver, and gonads, to assess their potential bioaccumulation in fish and human health risks. Phenolic compounds were found to be widespread in various freshwater environments throughout South Korea. Phenol was predominant in all matrices, with median concentrations of 57.0 ng/L in freshwater, 54.3 ng/g dry weight (dw) in sediment, and ranging from 71 ng/g wet weight (ww) to 621 ng/g ww in crucian carp tissues. Cresols were the second most dominant compound, with m-cresol exhibiting the highest prevalence. Most of the compounds detected in crucian carp samples were also detected in freshwater and sediment, whereas pentachlorophenol and 2,4,6-tribromophenol were exclusively detected in crucian carp tissues. A high bioaccumulation potential in the liver was observed for most phenolic compounds [median log bioconcentration factor (BCF): 3.2-3.7]. Interestingly, only m-cresol showed high bioaccumulation potential in the gills (median log BCF: 3.1). The estimated daily intake of phenolic compounds suggested that it does not pose an immediate concern for human exposure owing to crucian carp consumption. These findings enhance our understanding of the exposure status, distribution, and bioaccumulation potency of phenolic compounds in aquatic ecosystems and emphasize the importance of ongoing monitoring and risk assessment efforts.

3.13.P-Th213 Integrated Approaches to Testing and Assessment (IATA) for Bioaccumulation: A Surfactant Case Study

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Bioaccumulation assessment is a critical component of a chemical hazard and risk assessments. Under the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) framework, a Weight of Evidence (WOE) approach is recommended when performing bioaccumulation assessments to allow for the integration of multiple lines of evidence (LoE). An OECD Bioaccumulation Integrated Approach to Testing and Assessment (B IATA) was recently adopted, providing guiding principles for the collection, generation, evaluation, and integration of multiple LoE to inform bioaccumulation assessments. Assessing the bioconcentration of surfactants is inherently challenging due to their amphiphilic nature. For example, partitioning coefficients (e.g., logP) can be technically challenging to measure; however several experimental and predictive models are available. In this presentation, we use leverage the B IATA framework to assess the bioaccumulation potential of the anionic surfactant linear alkylbenzene sulfonate (LAS). Multiple LoE are integrated including various logP methodologies, in silico predictions, in vitro

clearance data, and standard in vivo fish bioaccumulation (OECD 305) results. We demonstrate how this framework can provide useful insight and transparent interpretation of bioaccumulation potential for surfactants across variations in alkyl chain length.

3.13.P-Th214 Metabolic Activities in Rainbow Trout (*Oncorhynchus mykiss*) S9 Fractions from Liver and Extrahepatic Organs as an Alternative In Vitro Ecotoxicity Assessment Approach

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Whole body biotransformation rate constants can be calculated by using appropriate in vitro to in vivo extrapolation (IVIVE) models. These models use in vitro intrinsic clearance CL in vitro, int rates derived with OECD Test Guideline (TG) 319A/B to estimate liver clearance rates in Rainbow trout, which are then extrapolated to a whole-body (in vivo) biotransformation rate constant. Primacyt GmbH supports this approach of alternative method to animal experiments.

Rainbow trout were maintained under controlled housing conditions according to OECD TG 319A/B. Specimens of eight sexually immature animals were obtained and pooled, including liver, gill, intestine, brain, heart and spleen to generate S9 fractions. These fractions were prepared to determine the Cytochrome P450 Phase I and Phase II enzyme activities by liquid chromatography-mass spectrometry analysis. Phase I activities were determined by using 7 compounds and glucuronidation and sulfation of 7-OH-Coumarin as Phase II reactions were analyzed as well.

Liver and intestine displayed the highest Cytochrome P450 activities of all organs tested. The Phase I formation of four metabolites (Acetaminophen, 1-OH-Midazolam, 4-OH-Diclofenac, and 6-OH-Chlorzoxazone) were linear up to 60 min in intestine and 120 min in liver. One metabolite (OH-Bupropion) was only detectable in liver with a moderate increase and two (4-OH-Mephenytoin and 7-OH-Coumarin) did not indicate a time-dependent increase or were not detectable. Extrahepatic Phase I activities of four enzymes were found in all organs with different specification to the tested compounds, three were not detectable. Phase II activities were detected in liver, intestine, gill and heart, but not in brain and spleen.

In summary, liver is the major organ for detoxification of compounds in Rainbow trout. Extrahepatic organs, mainly intestine, but also brain, heart, gill and spleen exhibit certain Phase I Cytochrome P450 activities with different specification to the reference compounds. Phase II enzyme activities were besides liver also detectable in intestine and gill. Our results suggest that extrahepatic organs, mainly intestine, should also be taken into account when bioaccumulation and in vitro clearance rates are determined for IVIVE modeling in Rainbow trout.

3.13.P-Th215 Can a Modified CaCo2 Assay Support the Assessment of Secondary Poisoning in Birds and Mammals?

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A new Birds and Mammals Guidance document was published by EFSA to revise the way of risk assessment for the registration of plant protection products (PPP). Trophic interactions through secondary poisoning assume the transfer of a PPP from the soil into the target organism, e.g. earthworms (1. Step) and then from earthworm into the mammal/bird after ingestion (2. Step). EFSA proposes a simplified but conservative approach by calculating the bioconcentration factor (BCF) value in the earthworm considering the logPow and predicted environmental concentration in soil and pore water. This equation leads to often unrealistically high BCF values. An alternative approach is a radioactive BCF study in earthworm, which can deliver data for the 1. Step of secondary poisoning process. The transfer from the earthworm to the bird and mammal remains unclear. An innovative alternative could potentially be the human CaCo2 assay, a well-established intestinal cell barrier model in human toxicology. We present here method development activities by adding the soil into the CaCo2 system to explore the transfer of substances in the context of secondary poisoning.

The gastrointestinal tract in the earthworm but also in the bird/mammal is an important barrier for absorption of PPP. CaCo2-cells are seeded on a transwell device, substance is applied to the apical compartment and the transfer over time to the basal compartment is measured by HPLC-MS to derive a Papp-value (cm/s). Substances were applied to two different soil types in different concentrations to allow an interaction between the soil and the substance. The prepared soil was applied afterwards to the CaCo2 assay. The addition of unsterile soil to the sterile CaCo2 assay was applicable under defined circumstances

(e.g. soil/buffer concentration, duration of the assay).

Different substances were tested in the assay. Orlistat, with high logPow, showed high adsorption to soil, while testosterone, erythromycin and acetaminophen with lower logPow showed low adsorption. Testosterone, acetaminophen and erythromycin showed the expected transfer rate also in this set-up, while Orlistat showed no transfer due to high adsorption rate. With this case study we showed promising first results to use the model also in an ecotoxicological context, like secondary poisoning. Future effort will expand the data set to better understand the applicability of the test system.

3.13.P-Th216 Legacy and Emerging Per- and Polyfluoroalkyl Substances (PFASs) in Tropical Marine Food Webs

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The bioaccumulation and trophic transfer of legacy and emerging per- and polyfluoroalkyl substances (PFASs) were determined within tropical food webs of the offshore zones in Singapore. Multiple aquatic organisms, surface water, sediments, soils and sea grasses were analysed for 44 PFASs (including long and short chain lengths). Stable isotope analyses of carbon and nitrogen were performed to elucidate the food web structure and trophic dynamics. Results revealed that PFASs were prevalent in all food web compartments. Perfluorobutanoic acid (PFBA) and perfluorobutane sulfonate (PFBS) were the predominant PFASs in water phase, while perfluorooctane sulfonate (PFOS) and perfluoroundecanoic acid (PFUnA) contributed most to the sum of target PFASs in sediments and marine organisms. Perfluoroalkyl carboxylates (PFCAs), GenX and perfluoroalkyl ether sulfones were potentially bioaccumulative with bioconcentration factors (BCFs) exceeding 2000. In addition, there was a significant positive correlation between BCFs and the carbon chain length of perfluoroalkyl carboxylic acids (PFCAs). This study emphasizes the importance to monitor PFASs in food webs to gain greater insight into the PFAS contaminations and thus provide complete evaluations of PFAS risk for humans through consumption of marine organisms.

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3.13.P-Th217 Trophic Transfer of Halogenated Polycyclic Aromatic Hydrocarbons in Benthos from Gyeonggi Bay, South Korea

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Trophic magnification factor (TMF) of traditional and halogenated polycyclic aromatic hydrocarbons (T-PAHs and Hl-PAHs) in a benthic web (8 invertebrates) was determined in Gyeonggi Bay, South Korea. Hl-PAHs included chlorinated-PAHs (Cl-PAHs) and brominated-PAHs (Br-PAHs). The nitrogen-stable isotope ratios ($\delta^{15}\text{N}$) were used to estimate the trophic position (TP) of invertebrates. The TP of invertebrates ranged from 0.90 to 3.43, with a mean of 2.29. 1-Bromopyrene, benzo[k]fluorine, and other high molecular T-PAHs (5 and 6 rings) were biomagnified through the whole food web (TMF > 1.2), while low molecular T-PAHs and other Hl-PAHs showed similar levels (TMF = 1.0) or were not biomagnified (TMF < 1.0). In particular, the trophic transfer of T-PAHs and Hl-PAHs was pronounced in the polychaetes and bivalves (e.g., *Perinereis nuntia*, manila clam, and oyster). The relatively lower accumulation rate in crabs with the highest TP appears to be due to the contribution of different food sources. The highest abundance of polychaetes and mollusks, which feed primarily on sediments and particles, suggests that food sources are a major factor in determining TMF in benthic ecosystems. However, the detection frequencies of Cl-PAHs and Br-PAHs in invertebrates are low (20.5% and 21.2%), making accurate data interpretation difficult, so more precise measurements and analysis of many samples are needed in the future. Overall, this study provided TPs of T-PAHs and Hl-PAHs in invertebrates and could be applied to determine the trophic transfer of contaminants in coastal food webs.

3.13.P-Th218 R-BIONIC – An R Package to Build Confidence in, and Drive Understanding of In Silico Predictions for Bioconcentration in Ionisable Compounds Using Toxicokinetics

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There is regulatory and societal drive to move away from traditional toxicity testing to animal-free new approach methodologies (NAMs). An essential part of NAMs is in silico tools able to deliver robust predictions used to support safety decisions. The BIONIC model by Armitage et al. (2013) is a toxicokinetic model designed to predict bioconcentration for a wide range of chemical groups, including ionisable compounds (IOCs) and quaternary ammonium compounds (QACs).

We present an R-package based on the BIONIC model. The package reviews and implements the mathematical model, building a complete view of the parameter space for the model and each sub-model, and structuring them as modules within the R package. We determined sensitive parameters for specific applicability domains/chemical groups and explored the robustness of the model through its R implementation (programmatically).

The main output is an open source, readily available R-package with accompanying technical documentation. The package mirrors the functionality of the previously published Excel/VBA tool for this model, with some enhancements such as a modern user interface written in Rshiny. The package implements modern software architectures and paradigms that enable robust and streamlined downstream analyses e.g. uncertainty and sensitivity calculations on predictions. The motivation for building the package is the hope that more users can further develop and contribute to the model for their own use cases facilitated by links to programmatic workflows and better integration with other R packages. Finally, we expect that the learnings and results from this work may help the modelling community build confidence and experience in the use of this model.

3.13.P-Th219 Validation and Application of the Multibox AQUAWEB Model for Trophic Magnification Factors of Volatile Methylsiloxanes

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Bioaccumulation assessment employs various metrics derived from quantifying chemical uptake and depuration rates. These metrics indicate the likelihood of a chemical accumulating within an organism and potentially biomagnifying up the food chain. For highly lipophilic compounds such as volatile methylsiloxanes (VMS), Trophic Magnification Factors (TMF) are especially important as it indicates a chemical's bioaccumulation behavior within a food web, considering all exposure routes and potential magnification effects. The objective of the current study is (i) to validate the Multibox-AQUAWEB (MBAW) model using measured concentrations of three cyclic VMS (D4, D5, and D6) and two polychlorinated biphenyls (PCB-153 and -180 as references) in biota from food webs in Yugawara coast, Japan and (ii) to predict TMFs of cyclic and linear VMS in real-world food webs. For model validation, Chi-square goodness-of-fit tests revealed good agreements between the modeled and measured concentrations, indicating the model's accuracy. Accordingly, the modeled TMFs of the cyclic VMS and PCBs were also in good agreement with the measured TMFs. For the food webs of Yugawara coast, the mean TMF of each cyclic VMS was less than 1 with strong or moderate statistical significance (p-value <0.10), suggesting trophic dilution, while the mean TMFs of PCB-153 and -180 exceeded 1 with strong statistical significance (p-value <0.05), indicating trophic magnification. The MBAW model was applied to the selected six food webs (Lake Erie, USA; False Creek, Canada; Lake Pepin, USA, Lake Ontario, USA/Canada; Oslofjord, Norway; and Tokyo Bay, Japan) to predict TMFs of cyclic and linear VMS. The mean TMF of each VMS in each food web selected was shown to be less than 1, indicating trophic dilution of VMS through dietary exposure pathways. An exception to this trend was observed for L5 in one food web, with a mean TMF of 1.1 (without statistical significance). In laboratory studies, measurement of the uptake and biotransformation rates of L5 was more challenging, resulting in greater uncertainty associated with the rates determined. Further research to generate more accurate uptake and biotransformation rates for L5 would enhance the model predictions. Overall, the MBAW model predicted that cyclic and linear VMS are likely to undergo trophic dilution in the selected food webs.

3.13.P-Th220 Evaluating Bioaccumulation Potential of Cyclic and Linear Volatile Methylsiloxanes: A Comprehensive Analysis Using the Bioaccumulation Assessment Tool

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The Bioaccumulation Assessment Tool (BAT, v2.02) collects and incorporates various lines of evidence (LoEs) related to the bioaccumulation of chemicals in both aquatic and terrestrial species. By using LoEs of bioconcentration factor (BCF), bioaccumulation factor (BAF), biomagnification factor (BMF), and trophic magnification factor (TMF), the process generates quantitative strengths of evidence, enhancing the comprehensive understanding of the bioaccumulation of compounds. This study reviewed 49 published articles and laboratory reports on fish (82%) and terrestrial species (18%). The objective was to

perform a comprehensive analysis of the bioaccumulation of three cyclic (D4, D5, and D6) and five linear (L2, L3, L4, L5, and L6) volatile methylsiloxanes (VMS) in biota. A total of 167 bioaccumulation values were extracted, underscoring the significance of multiple outcomes of bioaccumulation metrics. Considerable variability of BCF and BAF values was observed for VMS in aquatic organisms, which ranged from below to above their respective threshold values. The strength of evidence for non-bioaccumulation was 750% for D6, L2, L5, and L6 based on BCF and BAF while the other VMS compounds showed weak strength. Conversely, laboratory BMF values were generally less than 1 (i.e., the strength of evidence for non-bioaccumulation >67%), indicating low bioaccumulation through dietary uptake. Field BMFs displayed more variability but still suggested a low bioaccumulation potential. For the bioaccumulation assessment in terrestrial species, the study examined several physiologically based toxicokinetic and in vitro-in vivo extrapolation studies and derived BMFs of VMS in rats. The derived and predicted BMFs for terrestrial species are relatively small (less than 0.153), suggesting a low bioaccumulation potential. All field BMFs in terrestrial species were less than 1. This is primarily because all VMS are predominantly depurated via respiratory elimination. According to a single field TMF study for air-breathing species, D4 demonstrated trophic dilution in the terrestrial food-web, while no definitive conclusion was reached for D5 and D6 regarding either trophic magnification or dilution. For a better bioaccumulation assessment of VMS, comprehensive surveys of spatial chemical exposures, biota home range, and sampling locations are needed to address the inherent variability in environmental conditions contributing to uncertainties in the bioaccumulation assessment.

3.13.P-Th221 The Freshwater Amphipod *Gammarus Fossarum* as European Model Species for HYBIT Application: Review from Literature and Case Study

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The *Hyalella azteca* Bioconcentration Test (HYBIT) provides a non-vertebrate alternative to fish bioconcentration studies for determination of bioconcentration factor (BCF) as part of the regulatory chemical safety assessment of pollutants in aquatic ecosystem (REACH regulation). HYBIT test guideline focused on the North American amphipod *H. azteca*, but it also includes the possibility to work with other amphipod species. Here, we aim at highlighting the interest for including the European freshwater amphipod *Gammarus fossarum* as model species in HYBIT test guideline. First, we reviewed the literature to compare BCF values that are already available for *Gammarus* sp. and *H. azteca*. We found that BCF in both models are strongly correlated. Then, as a proof study of feasibility, an aqueous bioconcentration experiment in semi-static conditions was conducted with *G. fossarum* to assess the kinetics of bioaccumulation and depuration of prochloraz (fungicide of the imidazole family) and phenanthrene (polycyclic aromatic hydrocarbon) by following HYBIT protocol. HYBIT criteria validity were respected and BCF values were determined for both molecules. We thus demonstrate the applicability of HYBIT protocol to *G. fossarum*. Altogether, both review from literature and case study will support an interlaboratory assay project planned in 2025 with several European countries that aim to include gammarids as a second amphipod model in HYBIT test guideline.

3.13.P-Th222 From Plastic Debris to Sea Turtles: A Comparative Analysis of Plastic Additive Profiles

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Sea turtles are key bioindicators of chemical pollution due to their long lifespans, slow digestion, high trophic level, and frequent plastic ingestion. Plastic additives, which enhance plastic properties, can leach easily into the environment and cause health impact. However, research on these additives in marine top predators, including sea turtles, and their links to diet and plastic ingestion remains limited. We investigated plastic additives and polychlorinated biphenyls (PCBs) in the livers of 20 sea turtle carcasses, including 9 loggerheads (*Caretta caretta*) and 11 green turtles (*Chelonia mydas*), along with nine types of plastic commonly ingested by sea turtles. The target chemicals included PCBs, hexabromocyclododecanes (HBCDs), antioxidants, phthalate esters, UV stabilizers, and tire related additives. The accumulation pattern in sea turtles was phthalate esters > UV stabilizers > antioxidants > tire related additives > PCBs > HBCDs. Plastic additives were widely detected in the liver tissues of both species.

Interestingly, green turtles showed significantly higher levels ($143 \pm 93 \text{ ?g/g lw}$) than loggerheads ($92 \pm 35 \text{ ?g/g lw}$, $p < 0.05$). Green turtles also had higher levels of antioxidants and UV stabilizers, though not significantly. This finding is notable given green turtles in this study ($72 \pm 22 \text{ n/ind.}$) ingested more plastic than loggerheads ($40 \pm 24 \text{ n/ind.}$, $p > 0.05$). In contrast, loggerheads accumulated more PCBs ($54 \pm 199 \text{ ng/g lw}$) than greens ($8.5 \pm 8.1 \text{ ng/g lw}$), reflecting their omnivorous diet and greater PCB exposure through the food web. Plastics showed high concentrations of phthalate ester (DEHP: $11,061 \pm 6,504 \text{ ng/g dry weight (dw)}$), followed by antioxidant (Irganox 1076: $816 \pm 550 \text{ ng/g dw}$). Phthalates were predominant in several plastics (e.g., black plastic bag), implying that plastic additives in sea turtles are linked to plastics they commonly ingest. In contrast, antioxidants were dominant in other plastics (e.g., food packaging), but their lower accumulation in sea turtles may reflect their relatively low bioconcentration potential. This study underscores the significant accumulation of plastic additives in marine wildlife, particularly sea turtles, surpassing traditional POPs like PCBs. While further analysis of prey is needed for confirmation, we suggest that plastic ingestion may play a key role in the transfer of plastic additives.

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3.13.P-Th223 Evaluation of Bioaccumulation Thresholds for Aquatic Invertebrates and Fish Relative to Different Environmental Protection Goals

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The assessment of chemical bioaccumulation (B) in aquatic organisms is a critical component of global chemical assessment and regulatory programs. A metric typically used to describe a chemical's B potential is the bioconcentration factor (BCF), which is commonly measured in the laboratory following aqueous exposures with fish. However, because fish BCF tests require large numbers of experimental (vertebrate) animals, there has been increased efforts to develop new approach methodologies (NAMs) for B assessment. An example of one of these efforts is the non-vertebrate *Hyaella azteca* BCF test (i.e., "HYBIT"; OECD TG 321). Although the HYBIT has been adopted for regulatory assessments in Europe, it is unclear if B thresholds derived from fish data are appropriate for aquatic invertebrates. Between these two taxa there are notable differences in their physiology (e.g., biotransformation capacity, lipid content) and ecology (e.g., trophic position, lifespans, feeding habits) which influences chemical uptake and elimination. Historically, B criteria developed under the Stockholm Convention and other regulatory jurisdictions were established to prevent biomagnification in higher trophic level species and across food webs. The adoption of an invertebrate BCF test suggests that regulatory B protection goals have shifted from preventing B in higher trophic level species to avoiding accumulation in species living at the base of food webs. It is therefore important to assess and compare accumulation patterns in aquatic invertebrates relative to those expected in higher trophic level fish species. Here we employ empirical and model-based assessments to compare BCFs in *H. azteca* with B metrics derived in fish, examine biological, chemical, and ecological conditions that influence derived B metrics (i.e., BCFs, biomagnification factors [BMF], and trophic magnification factors [TMF]), and evaluate where there may be differences in accumulation between these two taxa. Proposals will be developed to support the robust use of bioaccumulation metrics derived for aquatic organisms in scientific and regulatory contexts.

3.13.P-Th224 Advancing Ecotoxicological In Vitro Testing: Comparative Evaluation of Trout Liver Metabolic Assay (OECD TG 319 A & B) and the *Hyaella azteca* Bioconcentration Test (HYBIT, OECD TG 321) Bioassays

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The development of reliable and relevant in vitro assays for ecotoxicology is crucial for reducing dependency on whole-organism bioassays while improving the accuracy of chemical hazard assessments. We discuss the advantages of the trout in vitro metabolic assays over the *Hyaella azteca* (HYBIT) bioassays in evaluating xenobiotic metabolism and Bioaccumulation prediction. Liver S9 fractions and hepatocytes offer a mammalian-based metabolic model incorporating phase I and phase II enzymatic pathways, enabling detailed insight into xenobiotic biotransformation. In contrast,

Hyalella azteca bioassays, while ecologically relevant, are limited by their inability to provide mechanistic understanding of metabolic pathways and are resource-intensive in terms of time, organismal use and cost.

Our comparative analysis revealed that liver S9 and hepatocytes metabolism assays not only reduction of organisms used but also allowed for high-throughput screening and quantitative assessment of metabolic kinetics. The *Hyalella azteca* assays, while valuable for whole-organism exposure studies, were less sensitive to detecting sub-lethal metabolic perturbations and lacked the resolution provided by biochemical assays.

By integrating trout liver S9 and hepatocytes assays into the workflow, researchers can achieve a more efficient and mechanistically informative approach to chemical hazard assessment. This aligns with global initiatives to refine and reduce whole-organism testing while enhancing predictive accuracy for bioaccumulation in ecological risk assessments.

3.13.P-Th225 Integrated Approaches to Testing and Assessment (IATA) for Bioaccumulation: Fragrance Ingredients as Case Studies

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As part of national and international regulatory programs, the assessment of the potential for a chemical to bioaccumulate is an essential endpoint e.g. as part of PBT (persistent, bioaccumulative and toxic) assessment of chemicals. A weight of evidence (WoE) approach is recommended by most regulatory jurisdictions, such as REACH. However, there is often no clear strategic guidance for implementing this. For the purposes of B assessment, multiple lines of evidence (LoE) can be used to quantify, measure, and qualify the common metrics of the e.g. bioconcentration factor (BCF). LoE can comprise laboratory or field data; in silico predictions; and combinations of in vitro and in silico data. An Integrated Approach for Testing and Assessment (IATA) for Bioaccumulation has recently been published (OECD) encompassing these LoE, in order to facilitate the process for evaluators to collate, generate, integrate, evaluate, and compare LoE for clear and transparent decision making in B assessment.

Here we demonstrate the applicability of the IATA to combine different LoEs for the bioaccumulation assessment of fragrance ingredients: Musk Xylene, Opalal, 8-Cyclohexadecen-1-one and Cyclohexyl Salicylate. LoEs include in silico predictions of log Kow values, measured log Kow values, predicted biotransformation half-lives, in vitro intrinsic clearance measured in the trout liver S9 fractions substrate depletion assay (OECD Test Guideline (TG) 319B), predicted bioconcentration factors (BCF) using QSARs and measured BCFs in fish (OECD TG 305).

These case studies demonstrate how multiple LoEs can be integrated for bioaccumulation assessment of data poor and data rich fragrance ingredients, illustrating how this tiered assessment approach can be effectively and transparently applied to prioritise / de-prioritise the need for higher tier B testing.

3.13.P-Th226 Intelligent Sampling in Standard In Vivo Toxicity Studies to Complement Bioaccumulation Assessments in Mammals – Fragrance Ingredients as Case Studies

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Traditionally, a fish bioconcentration factor has been employed to determine the propensity of chemicals to bioaccumulate in organisms in the environment. The requirement to perform a separate assessment of bioaccumulation in terrestrial air-breathing species was introduced in the 2017 revision of the Guidance on Information Requirements and Chemical Safety Assessment, however without technical guidance on how to develop/achieve such an assessment, beyond partitioning-based screening criteria. A tiered assessment approach was proposed in an ECHA guidance document; i.e. in silico screening, in vitro biotransformation assays, in vivo mammalian studies as a last option.

In the two case studies presented here, a smart sampling approach within standard in vivo repeated dose toxicity studies required from Annex VIII and above in REACH was applied for two fragrance ingredients, Ambermax and Glycolirral. The OECD TG 421 Reproduction/Developmental Toxicity Screening Tests were carried out as a REACH requirement. Rats were exposed daily for 28 days by oral gavage. Plasma, urine, faeces and organs (liver, kidney), which are routinely collected at the end of chemical exposure were used for analytical screening. In addition, adipose and muscle tissues were collected from the same animals. Plasma samples were collected at 0 h, 0.5 h, 2 h, 4h and 24 h post dose at day 1 and at day 28. Plasma and tissue samples were analysed by GC-MS and LC-MC for parent chemicals and potential metabolites from the vehicle control group, low and mid dose.

Rapid metabolic transformations of Ambermax and Glycolieral were observed with peak plasma concentrations at 2–4 h post dose. Hydroxylated and glucuronidated metabolites were detected for both chemicals. Concentrations in adipose tissue were dose dependent and in the low ng/mg tissue range, indicating that these rapidly metabolized molecules did not bioaccumulate in the fat tissue.

By integrating additional samplings without requiring additional animals in line with the 3Rs, data from standard repeated dose studies can be leveraged to develop further weight-of-evidence with respect to body distribution (in particular adipose tissue) and elimination half-lives of chemicals. Combining data from in vitro intrinsic clearance in S9 fractions with IVIVE modeling will allow to use these case studies of highly hydrophobic chemicals to model bioaccumulation based on in vitro data.

3.13.P-Th227 Exploring the Potential of Bioconcentration, Bioaccumulation and Trophic Transfer of Pesticide Mixtures in Freshwater Ecosystems

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Freshwater ecosystems, especially those near agricultural fields, are highly susceptible to complex pesticide mixtures that can harm aquatic organisms. One of the key challenges in ecotoxicological risk assessment is predicting how organisms will respond to exposure to multiple pesticides simultaneously. As part of the H2020 SPRINT project, this study aimed to tackle this challenge by assessing the ecotoxicity of pesticide mixtures on aquatic organisms using a high-tier evaluation approach, employing a three trophic level aquatic microcosm. The experimental design included a trophic chain model with a producer (*Nasturtium officinale*), a primary consumer (*Atyaephyra desmarestii*), and a secondary consumer (*Procambarus clarkii*). This setup enabled the evaluation of pesticide bioconcentration, bio-amplification, and transfer across trophic levels. We tested two pesticide mixtures (five pesticides each) based on the pesticides found in aquatic systems of two case studies (Spain and Czechia) under two conditions: control and predicted environmental concentrations (PEC). Bioconcentration tests showed significant pesticide accumulation in the three aquatic organisms from both Spain and Czechia, with *N. officinale* accumulating the most. Overall, organisms in Czechia had higher pesticide levels. *N. officinale* consistently had the highest bioconcentration factors (BCFs), acting as a pesticide reservoir, while BCFs decreased from *A. desmarestii* to the *P. clarkii*, suggesting reduced accumulation at higher trophic levels. The pesticides transfer across trophic levels showed that the pesticides levels in the producer directly influenced the levels ingested by the primary consumer and that the highest bioconcentration occurred when the primary consumer ingested a contaminated producer while exposed to the PEC, highlighting the importance of both contaminated food sources and pesticide mixtures in the environment. The biomagnification factors were consistently low across both countries, indicating minimal trophic transfer of pesticides from primary to secondary consumers. These findings suggest that while primary producers are at greater risk for pesticide toxicity, the overall risk of bioaccumulation and biomagnification within this food chain is low. Further research is crucial to gain a deeper understanding of the ecological impacts of pesticide mixture bioaccumulation and to assess its wider effects on biodiversity and ecosystem health in aquatic environments.

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3.13.P-Th228 Crayfish Behavior in the Shadow of Bioaccumulation of Ionizable and Neutral Pharmaceuticals

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Ionizable environmental pollutants, including pharmaceutical residues, can be readily bioavailable, bioaccumulative, and potentially toxic for aquatic biota. Risks for exposed organisms arise from continuous exposure, accessible ion entrance through cell membranes and various modes of action on cell receptors. In the present study, we determined behavioral changes in marbled crayfish *Procambarus virginalis* exposed to environmentally relevant (EC) and lowest observed effect concentrations (LOEC) of three selected pharmaceuticals. Anticonvulsant carbamazepine (neutral compound; EC =11.6; LOEC = 116 µg/L), anti-inflammatory diclofenac (ionizable weakly acidic compound; EC =31; LOEC = 310 µg/L), and antihistamine diphenhydramine (ionizable weakly basic compound; EC =1.4; LOEC = 95 µg/L) were selected to investigate pharmaceutical-induced behavioral alterations in crayfish after the 96-hour exposure under laboratory conditions (water pH 7.6 and temperature 22°C). The results revealed that neutral carbamazepine caused alterations in crayfish behavior, while ionizable diclofenac and diphenhydramine showed insignificant changes. We observed almost twice the distance moved and walking rates in crayfish exposed to EC carbamazepine compared to solvent control. However, carbamazepine exposure LOEC did not show any difference in the crayfish distance moved or walking rate. All test pharmaceuticals on both EC and LOEC levels evoked increments in movement activity, but only carbamazepine exposure elicited a significant effect. This study clarifies that physico-chemical properties and modes of action of the test pharmaceutical compounds could play important roles in how aquatic crustaceans distinctly cope with chemical stressors. Changes in ecologically important behavioral traits can potentially arise from exposure to environmental pharmaceuticals.

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3.13.P-Th230 *Hyalella azteca* Bioconcentration Test (OECD 321) - Optimisation and Automatisations Guillaume Cottin¹, Cyril Sweetlove¹, Celine Le Cocq¹, Mahily Cereyon¹, Alaa Bensetra² and Rita Castor², (1)L'Oréal Research & Innovation, France, (2)L'Oréal Research & Innovation Analytical Sciences Department, France

In the context of the Animal Testing Ban (ATB) regulation for cosmetics sector, in vivo tests involving laboratory animals (OECD 305) cannot be carried out. The publication of OECD 321 using a freshwater amphipod supports a robust bioaccumulation assessment of cosmetic ingredients¹. Performing this test brings technical difficulties and requires expertise in both biology and analytical chemistry.

The aim of this study is to present optimized and automated methods for performing the bioaccumulation test according to OECD guideline 321 and aims to present the best practice guidance for assessing the bioaccumulation of cosmetic ingredients.

Performing an OECD 321 test (HYBIT) in the laboratory requires rearing *Hyalella azteca*. Although *Hyalella azteca* is an easy organism to breed under laboratory conditions, it requires suitable infrastructures and a considerable amount of human time. Depending on the size of the farm, preparation of culture media, maintenance and feeding can monopolize up to 50% of a technician's time.

As the HYBIT test is only carried out on male hyalella, it is necessary to carry out a sexing sort prior to the bioaccumulation test. Added to this is the optimization of the analytical monitoring method (in water and in *Hyalella azteca*) as well as ecotoxicity pre-testing to define the target exposure concentration. These constraints limit testing capacities and make it difficult to carry out environmental assessments of a global product portfolio in a short space of time.

Optimizations and automations have been developed by L'Oréal to scale up OECD 321 testing capabilities and reduce operator-dependent biological variability.

These developments mainly concern the semi-automation of hyalella breeding and the development of a sorting robot. The limiting step remains the development of an analytical method.

Advances in the development of alternative methods provide a toolbox to correctly assess the

bioaccumulation of chemical substances placed on the market. B criteria assessment should follow a multi-source data approach, integrating models, in vitro biotransformation data and monitoring studies, for a more accurate assessment of the bioaccumulation potential of hydrophobic substances. These developments on the optimization and automation of the OECD HYBIT 321 test support and promote a NAMs methodology for bioaccumulation assessment, particularly in a regulatory context that prohibits testing on laboratory animals.

3.14 From Persistence to Mobility: Integrating Science with Policy and Bridging Gaps in Understanding Impact and Mobility

3.14.T-01 Scoping the Limitations of Koc to Assess Mobility of Acidic Pesticides

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The mobility of pesticides is evaluated by studying the sorption affinity these chemicals have towards soil or sediment particles. A low sorption constant for organic carbon (Koc) of less than Log 2 indicates high mobility and potential for surface and groundwater contamination. However, in contrast to neutral chemicals, the sorption of ionizable pesticides is additionally influenced by environmental conditions such as pH. In the case of neutral pesticides at soil-relevant pH values (i.e., pH 4-9) approaches to determine Koc have been developed. Nonetheless, the behavior of compounds that are permanently charged or ionizable is not consistently taken into account in assessing their environmental mobility. Based on this, the aim is to understand how logKoc varies between ionizable and non-ionizable pesticides, depending on ionization.

Pesticide characteristics (Koc or Kfoc, pKa, acid or base indication, and dissociation) were obtained from the Pesticide Properties DataBase. The charge state for the ionizable chemicals was determined and the experimental values of Koc or Kfoc were obtained from the peer-review risk assessment reports of the European Food Safety Authority. From this data recompilation, the minimum logKoc and maximum logKoc were calculated to derive a Δ logKoc to evaluate the variation in sorption behavior of acidic, basic, and neutral pesticides.

The charged anionic compounds exhibit great Δ logKoc variability ranging from 0-1.7 indicating substantial variation in their sorption affinity. Their mobility is highly influenced by the charge state of the pesticide and also by environmental factors like pH and the charged state of different soil constituents. This also applies to chemicals that may be partly anionic and neutral ($5 < \text{pKa} < 7$), since a high variability of Δ logKoc is observed but with a slightly narrower range. For the cationic and non-ionizable chemicals, more consistent Δ logKoc are observed with relatively narrow ranges indicating relatively stable sorption affinity.

In conclusion, the high variability of Δ logKoc for anionic and ionizable acids indicates the need for pH-sensitive methods to evaluate their mobility in the environment. Due to the charge of the acidic compounds, these show weak interactions with organic matter and charged particles. Their sorption affinity may vary in different soil types which makes average Koc more uncertain for risk assessment.

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3.14.T-02 Weight of Evidence in the Persistence and Mobility Assessment of Data-Rich Substances

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PMT and vPvM will be included as new hazard classifications in the EU for the labelling of chemicals within CLP. The criteria for persistence (P) and mobility (M) have been defined and technical guidance

has been developed. In the regulation and the guidance it is foreseen that (i) all available data should be considered and (ii) a weight of evidence (WoE) approach should be applied.

Compared to many other classes of chemicals, plant protection products (PPP) are data rich concerning information about their behaviour and fate in the environment. This is due to extensive data requirements in the PPP EU and national regulations in which the potential transport of PPP to drinking water sources such as groundwater or river-bank filtrate has been addressed for several decades.

Principles for WoE published by ECHA state that a combination of information from several independent sources is used to give sufficient evidence to fulfil an information requirement. This depends on (i) quality, (ii) reliability, (iii) consistency and (iv) relevance regarding the respective issue. Consequently, it should be possible to overrule a first-tier assessment based on conservative simple standardized key studies by sufficient evidence from more reliable and realistic higher tier studies if these provide alternative or complementary information.

In the context of PPP registration many additional information sources and higher tier studies are available which should be used for a WoE approach. Since used in authorization of a PPP these studies have all or most of the following characteristics which justify their consideration and adequate weighting in the WoE approach: (i) Performed according to current guidelines, guidances or well described frameworks, (ii) extensive documentation, (iii) high quality standards (e.g. GLP for experimental or Good Modelling Practice for modelling studies) and (iv) peer reviewed (EFSA, RMS, stakeholders and public).

Additional available information includes for instance field degradation, outdoor lysimeter, field leaching studies, kinetic evaluations, modelling of persistence and mobility, and monitoring. The information can either be used to derive parameters that can be directly compared with the respective criteria, or they can provide additional information on the substance properties. For example, if a substance is not found in drinking water sources this is a clear indication of non-mobility provided false negatives can be ruled out.

3.14.T-03 Enhancing Regulatory Implementation for PMT and PBT Substances: A Comprehensive Categorization Approach Using the NORMAN SusDat Database

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Following the addition of the PBT/PMT hazard class in the Classification, Labeling and Packaging (CLP) regulation, there is an urgent need to rapidly identify chemicals that will require priority work to confirm or refute these properties. In this work, we propose a categorization framework for identifying substances with persistence (P), mobility (M) and bioaccumulable (B) properties and facilitating the implementation of CLP regulation, which requires industrials to label substances as PMT or PBT substances of human and environmental concern. We applied the categorization methodology to a large dataset, the NORMAN SusDat database, which accounts for more than 120k substances. While generating comparable scores for hundreds of thousands of substances may seem daunting due to varying data availability, our methodology accounts for the data source and attributes the classification to different scenarios that reflect the data quality and quantity, offering a practical solution. The scenarios include a conservative one that selects the highest available score and an average scenario that calculates the average for the scores issued from modeled data. Finally, the robust scenario prioritizes experimental over predicted data. We summarize the three scenarios' categorizations by calculating a global score that considers the classification's severity, the risk, and the substance's data availability. Within the proposed methodology, we prioritized persistency, a shared property for two CLP hazard classifications. Substances classified as vP and toxic with a high and medium level of confidence have a higher global score than PB or PM substances. We assume that vP - also called extremely persistent - substances will sooner or later represent a hazard to the environment or human health. We ranked substances according to their global score and split them into different lists with specific action recommendations for each list, one being substances ready for assessment that includes over 20k substances. Finally, we mapped the substances uses for the assessment list and identified the economic sectors where vPvM/PMT or vPvB/PBT substances occur the most. This work is part of the EU project PROMISCES, which aims to protect the environment, human health, and natural resources from PMT and prevent their impact within the soil-sediment-water system.

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3.14.T-04 Groundwater Monitoring Insights of 101 PMT/vPvM and PFAS

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Persistent and mobile chemicals (PMs), including polar per- and polyfluoroalkyl substances (PFAS), pose significant risks to human health and the environment due to their mobility and limited removal by current water treatment technologies. Many PMs remain unregulated, leaving groundwater resources vulnerable to contamination and long-term exposure risks. This study monitored 101 PM chemicals across 180 groundwater wells in Germany, located in various land-use areas, to assess the occurrence and distribution of toxic PMs and their regulatory coverage gaps. Samples were characterized based on hydrological and contamination factors, with analysis using a validated multi-target super-critical fluid chromatography method. High detection of toxic PMs was found. For example, 69% of the total abundance in groundwater was from toxic PMs, with polar PFAS and other organic/inorganic PMs frequently detected at high concentrations. Over 90% of detected PMs were classified as non-persistent under REACH, since aquifers are not considered in persistence testing. This classification fails to address their potential accumulation in groundwater. In addition, mobility and persistence metrics were limited. Standard metrics, like logK_{oc}, showed limited correlation with PM occurrence, whereas emission data better predicted PM abundance, suggesting that emission likelihood should be prioritized in regulation. The findings underscore the need for updated regulatory frameworks that consider emission potential, transformation products, and more realistic environmental compartments to effectively manage PM chemical risks in groundwater.

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3.14.T-05 Scoping Unregulated Pollutants in Aqueous Effluents for Circular Water Use in the Netherlands

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During periods with limited precipitation, the indirect reuse of treated municipal effluent via surface water is inescapable in the Netherlands. However, treated municipal effluent contains organic micropollutants (OMPs) such as pharmaceuticals, personal care products, pesticides, and industrial chemicals such as PFAS. Many of these OMPs may be persistent, mobile, and toxic substances (PMTs). EU laws such as the Urban Wastewater Treatment Directive (recast) and policies such as the Circular Economy Action Plans, promote the reuse of treated effluent. Regulation 2020/741 on the minimum requirements for water reuse creates a further legal framework for the reuse of effluent for agricultural irrigation. Despite these regulations, only a few OMPs are well regulated for effluent reuse. Thus, many PMTs and other OMPs may currently fall through the regulatory cracks and may not be sufficiently regulated for safe effluent use.

The Dutch emission registration database WATSON was used to identify OMPs emitted in Dutch effluents that could end up in the environment. The OMPs were grouped per use category (industrial chemicals, pesticides, personal care products, pharmaceuticals and other) and per regulation (EU, Dutch or not regulated). In 2014-2019, over 1000 micropollutants were detected in Dutch effluents. When comparing the EU and the Dutch regulations both regulatory groups have overlaps, but are still missing regulations for most industrial chemicals, pesticides, pharmaceuticals, and personal care products in the context of effluent reuse. Many PMTs and other OMPs are not regulated in Dutch effluents, neither by EU nor Dutch regulation. For the safe reuse of effluent in the Netherlands there is still a need for a regulatory framework for effluent quality. Such future new regulations may be based on exposure scenarios and use profiles (e.g. industrial chemical, pesticide, pharmaceutical, personal care product) or based on chemical structure, in contrast to the currently predominant compound-by-compound approaches.

3.14.P From Persistence to Mobility: Integrating Science with Policy and Bridging Gaps in Understanding Impact and Mobility

3.14.P-Tu236 Is the Organic Carbon Normalised Partition Coefficient the Most Appropriate Measure of Partitioning for Substances that are Highly Mobile in the Environment?

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Screening level assessment approaches based on log K_{OC} have been used to identify substances that could potentially meet previously defined criteria for the mobility of substances. Due to the lack of

suitable alternative metrics these prioritisation exercises have generally used the Organic Carbon normalised partition coefficient KOC as the indicator of substance mobility in the environment. However, reasonable concerns have been raised about the appropriateness of the KOC concept to indicate to potential for substances to be highly mobile in the environment because, whilst organic matter plays a dominant role in the adsorption of hydrophobic substances to surface soils, it is of considerably less importance for some ionisable substances and because organic matter is a much less important component of subsoils and other sub-surface media than it is in surface soils. Scientists and regulators will need to evaluate whether the KOC parameter is an appropriate descriptor for the partitioning of these highly mobile substances. However, whilst an empirical overall partition coefficient may seem like an appropriate measure the fact that soils, and other sub-surface media are highly variable in their composition, not just in relation to organic matter contents, but also the levels of iron and aluminium (oxy)hydroxides and clay minerals, as well as the many different types of clay minerals, empirical partition coefficients can be highly variable for the same substance in different soils and sub-surface media. The determination of partition coefficients in a standardised test medium could address the issue of variation between different media, although the selection of an appropriate medium composition that is adequately representative of the most relevant environmental media would be problematic. However, the OECD 121 screening test to determine KOC values effectively provides a single test medium in the form of a chromatography column to produce results that are directly comparable between different substances. There are a number of important limitations to the use of the Organic Carbon Normalised Partition Coefficient (KOC) for highly mobile substances, but there are also considerable difficulties associated with defining an appropriate alternative metric that could be used to define the relative mobility of different substances in a diverse range of environmental media.

3.14.P-Tu237 Fate and Transport of Biosolid-Derived PFAS across the Terrestrial-Aquatic Continuum of Agroecosystems

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Per- and polyfluoroalkyl substances (PFAS) are a highly diverse class of contaminants of emerging concern produced intentionally and unintentionally over the past 80 years with an ever-expanding inventory of thousands of unique known structures. Some PFAS have been linked to adverse human health effects, and many are known to bioaccumulate and biomagnify in terrestrial and aquatic species. The application of biosolids (i.e., sludge from wastewater treatment facilities) as a soil amendment on farmland is a widespread practice in the United States (US) and worldwide. While beneficial for soil health and sustainable waste management, this practice may represent a potentially concerning yet understudied pathway for PFAS contamination of soil and water. This study investigates PFAS fate and transport in soils and a freshwater stream on a working farm in Pennsylvania, US with 24 years of regular biosolid use. In January 2023, we began monitoring PFAS concentrations in surface soils (0-15 cm), stream sediments, and stream water using USEPA Method 1633. Preliminary results showed the detection of PFBS, PFOA, PFOS, PFNA, PFDA, PFDS, PFUnA, and PFDoA in soils, with total PFAS concentrations averaging 20 ± 4 ng/g. In addition, soil PFAS concentrations have remained constant over the last 1.5 years since biosolid application ceased. Compounds detected in the stream water differed from those found in the soil and included PFBA, PFBS, PFPeA, PFHxA, PFHxS, PFHpA, PFOA, PFOS, and PFNA, with total PFAS concentrations averaging 126 ± 25 ng/L. Interestingly, despite the presence of various PFAS in the water column, PFAS concentrations in sediments were below the quantification limits for most compounds. This may be related to the low organic matter content of the stream sediments (0.5-1%) and the high hydrophilicity of most compounds detected in the water column. Our results suggest that short-chain PFAS are more readily mobilized from the surface soil to the stream water. Future work will explore high-frequency sampling of stream water and sediments during high-flow conditions to investigate the role of storm events in mobilizing (long-chain) PFAS from the soil to the stream via overland flow. Altogether, these results will improve our understanding of PFAS fate and transport in agroecosystems and the potential implications for human and environmental health.

3.14.P-Tu238 The Leaching Calculator: A Tool for Predicting Leachability Within the Framework of Mobility in the New PMT/vPvM Hazard Class

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New CLP regulations highlight the importance of mobility assessments for classifying persistent and mobile substances, especially PMT (persistent, mobile, toxic) and vPvM (very persistent, very mobile) compounds. Traditional mobility assessments using the organic carbon partition coefficient (logK_{oc}) can be insufficient, as they overlook degradation processes, solubility, and other transport mechanisms. The proposed LeachingCalculator introduces a robust approach for predicting pesticide leachability to groundwater, complementing logK_{oc}-based evaluations.

The LeachingCalculator builds upon the established FOCUS PELMO model, simulating pesticide movement through soil using variables such as degradation half-life (DegT₅₀) and K_{oc}. By running simulations across representative European scenarios, the tool generates a leachability matrix, interpolating predictions for various combinations of DegT₅₀ and K_{oc}. The model's output includes conservative leachability thresholds (1% and 10%), classifying substances as non-mobile, mobile, or very mobile, aiding in regulatory decision-making.

Evaluation against groundwater monitoring data showed strong alignment between predicted mobility and observed contamination levels, although only 12 substances with sufficient data were analyzed. The LeachingCalculator is available as an open-source, user-friendly tool (<https://github.com/IMEDiman/LeachCalc>), enabling mobility assessments without requiring advanced modeling expertise. This approach ensures regulatory compliance while addressing critical gaps in current assessment frameworks.

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3.14.P-Tu239 Challenges in Assessing the Mobility of Specific Substance Groups

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A new CLP revision came into force in 2023 to better safeguarding drinking water resources from harmful substances. With the introduction of the new EU CLP hazard classes, the classification and labeling of chemicals now require the assessment of a completely new hazard property: the mobility of a chemical. This new hazard property focuses on the ability of substances to be transported with water through soil/sub-soils and potentially contaminate groundwater. Up to now, there is little experience in assessing the mobility of substances. The challenge becomes more complex with unstable or reactive substances. They may degrade or react with the soil before they have even reached significant depths. These substances may not follow the same leaching patterns as stable compounds because they break down in soil into transformation products. The mobility of these transformation products may differ from that of the parent compound, and understanding this difference is crucial for assessing the mobility of the parent substance.

Current experimental methods are limited as standard tests typically assume stability and do not consider in-depth how to assess the degradation or reaction of substances during their percolation through soils. The same applies to ionizable substances, which pose a unique challenge because their individual charge density can lead to interactions with soil particles, particularly soil organic matter and soil clay minerals. As such, their mobility in soil is highly dependent on the specific characteristics of the soil.

Existing test methods appear to be inadequate for reactive, unstable, or ionizable substances, and new testing approaches or adaptations seem necessary to ensure reliable prediction of chemical mobility. This poster highlights these challenges, emphasizing the need for scientific innovation and the importance of cross-sector information exchange.

3.14.P-Tu240 Issues Related to the Measurement of Log K_{oc} Using the OECD TG 121

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Recently, new hazard classes for Persistent, Mobile and Toxic (PMT) as well as very Persistent and very Mobile (vPvM) chemicals were included in the European Classification, Labeling and Packaging Regulation (CLP). The assessment information required for the evaluation of a chemical's Mobility (M) is the logarithm of the organic carbon-water partition coefficient (log K_{oc}). Multiple methods are available to measure this parameter, one of which is the OECD TG 121 which estimates the adsorption coefficient using High Performance Liquid Chromatography (HPLC). This method is a comparative approach with a number of standards being used to define the retention time in the column vs. their known log K_{oc} (as defined in the TG) and then estimating the log K_{oc} of the chemical of interest based on its retention time and using the regression established with the standards. However, this method was established over 20 years ago and would benefit from an update to take into consideration the latest scientific knowledge.

Indeed, this method has limitations, particularly for very mobile chemicals, as it is only applicable for substances with a log K_{oc} greater than 1.5. The accuracy of the results is also highly dependent on the choice of standards, which primarily represent Plant Protection Products and Hydrocarbons and do not encompass the diversity of chemicals in databases like REACH. Moreover, some of those standards are now restricted in their use and are difficultly accessible and others are ionizable, hence particular attention is required. Inconsistent log K_{oc} values for the same standards in the literature further raise questions about the method's accuracy. Additionally, there is a lack of proper standards covering the high end of the range. Finally, standards do not always elute in the expected order, complicating interpretation. To address these challenges, we have compiled data on log K_{oc} values and evaluated the variability introduced by different standards. We also propose alternative standards, particularly for fragrance materials, to improve the method's applicability and reliability across a broader range of chemicals.

3.14.P-Tu241 An Approach for Accounting for Structural Similarity in Reference Substance Selection for Highly Mobile Test Substances in OECD TG 121 and Similar Studies

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Adsorption or partition coefficients such as the organic carbon normalised partition coefficient are a key parameter for assessing the potential mobility of substance in the environment and is a key input into the Persistent, Mobile and Toxic assessment and very Persistent, and very Mobile assessment that are required to be conducted as part of EU Classification and Labelling regulations. An important guideline method for the determination of the K_{OC} value of a substance is the High Performance Liquid Chromatography method detailed in the OECD TG 121 guideline. The current OECD TG 121 guideline states that determined values using this method have improved accuracy if reference substances that are structurally related to the test substance are used for conducting the test. However, the guideline does not provide any further details regarding how structural similarity should be assessed or a potential approach for applying it in practice. Prior to being able to perform a structural similarity assessment, a defined list of substances that could potentially be used as reference substances needs to be compiled. The substances to be used as reference substances are required to have experimentally derived, reliable, partition coefficients.

Reference substances are included in the OECD TG 121 guideline. For highly mobile substances that cannot be tested under the OECD TG 121 guideline the retention times in the chromatography system used would need to be known so that reference substances with both shorter and longer retention times could be identified. The OECD QSAR Toolbox has been used to perform an initial assessment for ten substances identified in OECD TG 121 as reference substances to assess their structural similarity to a number of potential test substances. For the structural similarity profiler to operate, both a measure and a molecular feature are required to be selected; this assessment was performed using the Dice coefficient similarity measure and the PubChem molecular features system. Importantly, the results of this assessment demonstrate that the existing reference substances that are recommended for use in the OECD TG 121 test are not suitable reference substances for many of the substances that have been identified as potentially very mobile in the environment. Although the reference substances used may be suitable for diclofenac and benzamide, they are not suitable for EDTA, formamide, N,N-dimethylacrylamide, or sodium trichloroacetate.

3.14.P-Tu242 Finding the Right Way to Address the Potential Mobility – A Case of Fragrance Material

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New hazard classification for the European Union (EU) Classification Labelling and Packaging Regulation (CLP) were recently introduced implementing a new hazard class of Persistent (not or hardly degradable in the environment) Mobile (well soluble in water and therefore easily transported through the environment) and Toxic (toxic to ecosystem) - PMT as well as very Persistent and very Mobile (vPvM) chemicals. The combination of intrinsic P and M properties of chemicals enable them to be transported over long distances from the point of emission, pass natural and artificial barriers and enter drinking water sources causing a high concern with consequent exposure to humans and other organisms. To assess the potential of the material to be mobile, standardized methods have been developed and include estimating the adsorption/desorption behavior of chemicals on different soil types (OECD 106) and/or estimating an adsorption coefficient (K_{oc}) in soil and in sewage sludge using HPLC method (OECD 121). In an effort to understand and select the most appropriate approach for evaluating the potential Mobility of a material, a number of fragrance materials with various physical chemical properties will be selected and evaluated using both approaches (OECD 106 and 121). This will allow us to assess the applicability and challenges associated with current approaches when it comes to fragrance materials. In addition, the newly generated

data will be compared to Koc values generated by available models (EpiSuite) to evaluate the applicability to fragrance material s predictions.

3.14.P-Tu243 Development of a Screening Test for the Mobility of Polar and Ionisable Substances in the Environment

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The partitioning and binding of substances to components of soils and sediments is an important aspect of their environmental fate and mobility. Screening level assessment approaches to identify potentially mobile substances have generally been based on Koc, because organic matter plays a dominant role in the adsorption of many substances to surface soils. However, concerns have been raised about the appropriateness of the Koc concept to indicate the potential mobility of substances, principally because: (1) adsorption to organic matter is a less important adsorption mechanism for some ionisable substances; and (2) levels of organic matter in subsurface soils are much lower than in surface soils, so other interactions may be more dominant in those media. Currently, there is a lack of suitable alternative metrics by which the other interactions between substances and components of soils and sediments can be measured.

The OECD TG 121 screening method for the measurement of Koc on an HPLC system is often carried out as a simple, quick and cheap method that provides directly comparable results. The cyanopropyl stationary phase allows for the interaction of polar and non-polar groups of a test substance, but it does not include other interactions that may be influential in determining the adsorption properties of more polar and ionisable substances, including ion exchange interactions and some polar interactions.

It may be possible to extend the general concept of the OECD TG 121 screening test to consider additional interactions that occur in soils and other subsurface media, which are especially relevant for polar and ionisable substances. This work investigates whether state-of-the-art mixed mode stationary phases might have the potential to act as surrogates for surfaces where adsorption occurs in soils or sediments reflecting such interactions.

The most promising HPLC solid phases for the intended purpose were identified; we are performing tests with 30 substances using strong cation exchange (SCX), weak cation exchange (WCX), strong anion exchange (SAX), and weak anion exchange (WAX) HPLC-columns, each combined with reversed phase material. If the concept works, we will recommend how an extension of the OECD TG 121 could be set up experimentally. The results of the present project are intended to provide a possible solution and improve the mobility assessment of polar and ionic substances in low organic carbon soils and subsurface media.

3.14.P-Tu244 Mobility of Micropollutants that May Occur in Surface Water Used for Irrigation, in Soil

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Agricultural soil can be contaminated by various micropollutants. One of the sources of these contaminants is agricultural practice, where a variety of pesticides are used for plant protection. Another source of contamination can be river water that is often used for irrigation. River contamination depends on the sources of the compounds, that can be, for example, discharged wastewater, surface water runoff from agricultural or urban land, etc. Compounds occurrence in soils or their leaching from soil and migration towards groundwater depend on the climatic conditions, properties of the vadose zone environment and behavior of a particular compound, i.e., its sorption onto soils and sediments, and stability in the environment.

Based on our previous study focused on micropollutant monitoring in various matrices of intensively irrigated agricultural land, six compounds (1,3-diphenylguanidine, triethyl citrate, naphthalene-2-sulfonic acid, benzo(d)thiazole-2-sulfonic acid, 4-acetamidoantipyrine, and fluorinated telomer sulfonate) were

identified as potential contaminants that can occur in surface water and next in soil and ground water. Since they are found in the environment, they are relatively stable compounds. Their sorption in soil is significantly affected by their dissociation in the environment, i.e. by either negative or positive charges of their molecules.

In order to find out their behavior in various soil environments, we conducted standard sorption experiments for each of them in 16 representative soils of the Czech Republic. The Freundlich sorption isotherms were calculated to express relationships between concentrations in solution and sorbed onto soil particles. Multiple linear regressions were used to derive equations for predicting the Freundlich sorption coefficient (KF) using the properties of tested soils. These equations and the maps of soil properties were used to predict the KF value distributions within the irrigated areas and subsequently to delineate classes of compounds mobility in the soil environment. Next, specific groundwater vulnerability maps for each compound were obtained by combining the DRASTIC vulnerability index, mobility index and stability index.

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3.14.P-Tu245 Developing the New CLP Guidance on PBT/vPvB and PMT/vPvM Properties: Stakeholder Engagement, Key Considerations and Other Discussion Points

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Commission delegated Regulation (EU) 2023/707 of December 2022 has amended the CLP Regulation ((EC) No 1272/2008) to add new hazard classes and criteria for the classification, labelling and packaging of substances and mixtures. This regulatory action was dictated by the concern of substances possessing endocrine disrupting, as well as PBT (persistent, bioaccumulative, toxic), vPvB (very persistent, very bioaccumulative), PMT (persistent, mobile, toxic) and vPvM (very persistent, very mobile) properties. Related to the PMT/vPvM properties, the concerns refer to the substance's high persistence and mobility, the possibility to enter the water cycle, the potential for transport away from point sources, the ongoing emissions, build-up of environmental concentrations over time, combined with the potential toxicity. The new hazard classes introduced an urgent need to develop guidance on the application of the CLP criteria for these hazard classes. The European Chemicals Agency (ECHA) is managing the process of harmonised classification and labelling for substances and mixtures since the adoption of CLP in 2008 and has led the exercise of the development of CLP guidance for the new hazard classes. In the development of this guidance document, key ECHA stakeholders, namely Member States, the European Commission, Industry and NGOs were involved. The development of guidance on the assessment of PBT/vPvB and PMT/vPvM substances under CLP took place between early 2023 and the guidance publication date of November 2024. Important aspects on the structure of the guidance, the availability and relevance of different assessment methods, study-specific considerations, applicability and uncertainties will be presented for each individual property (P,B,M and T). Importantly, the necessary stages for conducting the weight-of-evidence determination will be presented, namely the collection of the available information, its evaluation and weighing, its integration and reporting, as well as description of the underlying uncertainty. Approaches regarding the combination of several experimental study results of the same study type will also be elaborated on, as well as example cases to specify further how all information lines can be integrated in order to come to a scientifically robust conclusion on the related CLP numerical criteria.

3.14.P-Tu246 From Policy Making to Implementing: First Lessons Learned from the Harmonisation of Classification and Labelling of Persistent and Mobile Substances Under CLP

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The Classification, Labelling and Packaging (CLP) Regulation ((EC) No 1272/2008) aims to ensure a high level of protection of human health and the environment, as well as the free movement of substances, mixtures and articles. The European Chemicals Agency (ECHA) has been managing the implementation of CLP via the process of harmonised classification and labelling (CLH), where proposals mainly from Member State Competent Authorities are submitted to ECHA for substances that may warrant a

harmonised classification. Commission delegated Regulation (EU) 2023/707 of December 2022 has amended CLP to add new hazard classes and classification criteria. Thus, as of the 20th of April 2023, proposals for harmonised classification can be submitted referring, among others, to a substance's PBT (persistent, bioaccumulative, toxic), vPvB (very persistent, very bioaccumulative), PMT (persistent, mobile, toxic) and vPvM (very persistent, very mobile) properties. Thus, an increasing number of proposals is expected to start entering the regulatory machinery of ECHA for these new hazard classes. The presentation will highlight key CLP considerations and criteria, with a focus on persistent and mobile substances, as well as the protection goal for the new hazard classes. It will introduce the main elements of the CLH regulatory process, including the various stages, timelines and actors. Importantly, it will summarise key messages and discussion points from the processing of the historical first cases on PBT and PMT properties that have been received between April 2023 and May 2025, when the SETAC meeting is due to take place. Relevant implications for Dossier Submitters/Competent Authorities will be elaborated upon that will tackle data availability and quality issues, the structuring of the presented information and the building of the overall weight-of-evidence, in order to come to a robust scientific conclusion when data is compared with the respective CLP numerical criteria. The issues that have been identified by ECHA during the initial stages of the processing of these harmonised proposals will be grouped and presented, aiming to increase transparency and clarity on regulatory processing and opinion-making. Additionally, elements relevant to the opinion-making process by ECHA's Risk Assessment Committee will be discussed.

3.14.P-Tu247 PBT/vPvB and PMT/vPvM as Hazard Classes in the Environmental Risk Assessment of Human Pharmaceuticals

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According to the draft Directive relating to medicinal products for human use the hazard classes persistent, bioaccumulative, toxic (PBT), very persistent, very bioaccumulative (vPvB) as well as persistent, mobile, toxic (PMT) and very persistent, very mobile (vPvM) should be evaluated according to the criteria of Annex I to the revised Classification-Labeling-Packaging (CLP) Regulation (EC) 1272/2008.

PMT/vPvM is a new hazard class that is currently being included in the CLP Regulation along with PBT/vPvB and endocrine active agents. Due to their low adsorption potential PMT/vPvM substances are negligibly retained by soil and sediment and pose a risk to surface water, groundwater and drinking water e.g. by leaching processes. PBT/vPvB substances accumulate in the environmental compartments and biota and can be distributed in the food chains. Ecotoxicological effects are strengthened by bioaccumulation and may also appear in remote areas like marine and polar regions.

The aim of the current research is to identify the PMT/vPvM substances in human pharmaceuticals being of concern for drinking water and groundwater. PBT/vPvB assessment was already specified in the environmental assessment of medicinal products from the outset and this was at best published in the European Public Assessment Reports (EPAR). For the new hazard class PMT/vPvM no assessment for human or veterinary medicinal products has been carried out to date. To get an overview, the German Environment Agency (UBA) evaluates the active substances in human medicinal products based on data from marketing authorisation procedures assessed in DE. From substances with complete data sets, data for just the half are published e.g. in EPARs. To date 10 human active ingredients were identified as PBT or vPvB. The proportion of identified PMT/vPvM substances is higher, but due to data gaps in many cases a definite PBT/vPvB or PMT/vPvM classification was not possible.

Veterinary medicinal products containing PBT/vPvB substances should generally not be authorised as per legal requirements; PMT/vPvM properties are not taken into account yet. For human active substances with PBT/vPvB or PMT/vPvM properties the draft directive relating to medicinal products for human use provides that a medical prescription will be required and the applicant shall sufficiently address the identified risks and propose risk mitigation measures to avoid or limit emissions into water, soil and air where indicated.

3.14.P-Tu248 Development of a Method for Analyzing Persistent, Mobile and Toxic Compounds, from Groundwater to Drinking Water, Using LC-HRMS

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The growing pressure on water resources is leading to drinking water shortages and increased pollution. Certain persistent, mobile, and toxic (PMT) substances in aquatic environments have intrinsic properties that allow them to remain in the environment, pass through filtration systems, and withstand conventional drinking water treatments. They can accumulate, especially when wastewater is recycled, eventually reaching resources intended for drinking water. Current data on PMT contamination in water resources are limited, raising significant environmental and public health concerns. In this context, this study focuses on analysing PM/PMT substances in groundwater, surface water, and drinking water of 4 locations around the world with a gradient of anthropic pressure on the water resources from no pressure a priori to increasing pressures depending on water treatment and reuse practices. The initial step of this study was to develop a sample preparation protocol and an LC-MS (liquid chromatography-mass spectrometry) method suited to these compounds.

A literature review of 480 PM/PMT molecules resulted in the selection of 59 compounds from various categories (pharmaceuticals, pesticides, and REACH-regulated chemicals) for targeted analytical method development keeping in mind that a broader suspect screening approach will be also conducted. To achieve an optimal sample preparation and analysis of PMT substances, five solid-phase extraction (SPE) protocols with different cartridges (Hydrophilic-Lipophilic Balance [HLB], Weak Anion Exchange [WAX], ENVI-Carb, Mixed-mode Anion Exchange [MAX], and Mixed-mode Cation Exchange [MCX]) were tested on the 59 selected compounds. For LC-MS analysis, four chromatographic columns (Biphenyl, BEHC18, Cortecs T3, and Acclaim Trinity P1) with various mobile phases were also evaluated.

Following evaluation, the Acclaim Trinity P1 chromatographic column demonstrated superior performances in terms of retention of polar analytes and sensitivity. Regarding sample preparation, 2 complementary methods were selected: HLB and WAX. The HLB cartridge achieved superior results, delivering higher recoveries and lower matrix effects for 43 compounds. Additionally, the WAX cartridge enhanced the extraction of 8 additional compounds that were insufficiently retained by the HLB cartridge. Application of this optimised method on real samples collected in the frame of project revealed the presence of various PM/PMT substances.

3.14.P-Tu249 Quantification of Prioritized Persistent Mobile and Toxic Substances in Urban Water of Barcelona: A Cross-Sectional Analysis of Rainfall, Runoff, and Infiltration Water

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Urbanization, climate change, and the increased frequency of extreme weather events are degrading urban water quality, necessitating adaptive water management strategies. Urban stormwater transports anthropogenic contaminants, including Persistent, Mobile, and Toxic (PMT) substances. These PMT substances pose significant threats to groundwater resources due to their persistence and mobility, with standard water treatments often failing in removal. Monitoring these substances is crucial to understand their fate in natural processes and to validate the mobility criterion used in prioritization. This study aims to deploy a robust analytical method using RPLC-ESI-TQ-MS/MS for the quantification of 40 selected PMT substances, prioritized by the German Federal Environment Agency (UBA) under REACH category A, in urban stormwater, groundwater, and Blue-Green Infrastructure (BGI) effluents, collected during two sampling campaigns, conducted during summer-fall 2024 and winter 2025, covering four districts across Barcelona city, with wells located above and below BGI recharge points. Samples undergo initial filtration and are enriched using vacuum-assisted evaporation before analysis. The method demonstrates high sensitivity and robustness, with chromatographic run times of 14 minutes, recoveries between 70% and 120%, relative standard deviations below 20%, and limits of detection below 10?ng/L. Preliminary results from the 2022 stormwater campaign revealed the detection of all 13 prioritized PMT substances in street runoff, with mean concentrations up to 1??g/L, and several in BGI effluents ranging between 0.1 and 1??g/L. The inclusion of groundwater samples in the current study allows for the exploration and evaluation of the extent of contamination resulting from runoff and BGI effluents in direct contact with the aquifer. Data analysis employs statistical methods to identify geospatial patterns and correlations with hydrological parameters and vehicular traffic. Human health risk assessments are performed for substances ubiquitous in groundwater and those with average concentrations exceeding 100?ng/L. This work underscores the significance of mobility in the environmental behavior of PMT substances and highlights the necessity of monitoring to inform management strategies. Understanding the sources,

transport, and fate of PMTs in urban water cycles is essential for protecting water resources and ensuring the safe reuse of groundwater.

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3.14.P-Tu250 Integrating Groundwater in the RAIDAR Environmental Fate Model for the Screening of PMT Chemicals

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Persistent, Mobile, and Toxic (PMT) and very Persistent and very Mobile (vPvM) chemicals are of concern to human and ecological health because of their potential for widespread exposure. Current hazard-based regulatory approaches, based on pass/fail bright-line threshold criteria, may not fully capture the exposure potential of these chemicals, potentially introducing biases in decision-making. This presentation outlines a tiered modelling framework to assess the environmental fate and human and ecological exposure of organic chemicals with potential PMT and vPvM concerns.

The Risk Assessment IDentification And Ranking (RAIDAR) model is a multimedia mass balance model combining environmental fate and aquatic and terrestrial bioaccumulation models to represent regional-scale environments. RAIDAR supports simulations for neutral and ionogenic organic chemicals (IOC). In this work RAIDAR was revised to include groundwater compartments increasing capacity to estimate how PMT chemicals may contaminate critical sources of drinking water. Groundwater conditions in the real world are quite variable; thus predictions of chemical transport to and from groundwater compartments are uncertain. The revised RAIDAR model allows users to select different assumptions (scenarios) including options for groundwater recharge from soil, freshwater, or freshwater sediments.

The results of different model assumptions were compared in a series of simulations using hypothetical organic chemicals encompassing a diverse range of chemical properties (e.g., log KOA 1-10, log KOC -2 to 10, neutrals and IOCs). The results reveal significant differences in multimedia mass distribution as a function of the chemical properties and for the different environmental configurations with mode of entry representing one of the most sensitive input parameters in the model calculations.

The revised RAIDAR model is included in the freely accessible Exposure And Safety Estimation (EAS-E) Suite online platform (www.eas-e-suite.com) to facilitate access and use by interested stakeholders. Future work could consider the refinement of a local scale fate model, such as RAIDAR Point Source in EAS-E Suite, which is more suitable for exploring the impacts of riverbank filtration on groundwater contamination.

3.14.P-Tu251 Development of Experimental Testing for the Mobility Assessment of Chemicals During River-Bank Filtration

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River-bank Filtration (RBF) is a simple and widely accepted technology which utilizes the natural filtration processes of subsurface materials to remove contaminants and improve the quality of the extracted water. However, certain non-degrading and highly mobile chemicals could be transported across RBF and contaminate the drinking water sources. In order to protect drinking water sources from such mobile chemicals, mobility criteria M has been proposed under EU REACH Regulation ((EC) No 1907/2006). The assessment of M criterion in this approach is based on the organic carbon normalised adsorption coefficient (KOC). In particular, the KOC approach does not account for I) sorption to clay and mineral surfaces, II) non-linear sorption (Freundlich isotherms), III) for aging processes, slow

adsorption to the inner particles, entrapment and irreversible sorption (non-extractable residues NER). Especially the sediments characteristics relevant for RBF in most cases are very poor of organic carbon and the adsorption process during bank passage could take weeks to months. Thus, the aforementioned interaction could become a key parameter for mobility considerations for RBF. This study aims to develop a column testing approach with aquifer-like material for the assessment of sorption processes during river-bank filtration accompanied by the modelling approach for evaluation. For this study, two aquifer materials with variable cation exchange capacity, content of organic carbon, texture (hydraulic conductivity between 10⁻³ and 10⁻⁵ m/s), and porosity will be selected. A column test setup with water saturation in up-flow condition similar to DIN 19528 (Leaching of solid materials Percolation method for the joint examination of the leaching behaviour of inorganic and organic substances) will be used as a basis for the development of this testing approach. Tests will be performed with three environmentally relevant test substances with different degree of polarities and at least one of them as ¹⁴C-radioactive labelled substance. An introduction to the test setup, selected reference substances and the first results obtained under this study will be presented.

3.15 Co-Occurrence of Contaminants in Urban and Rural Environments

3.15.T-01 Co-Occurrence of Pesticides and PFAS from Agricultural Pesticide Applications

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Per- and polyfluoroalkyl substances (PFAS) are frequently found in streams with most research focusing on urban sources of PFAS to surface water (e.g., firefighting foams, industrial releases, and wastewater treatment plant discharges). Pesticide applications are an under-investigated source of PFAS to streams in both agricultural and urban areas. Although many PFAS are not applied as pesticides there are concerns of increased PFAS levels in pesticide containers and as inert ingredients not listed on pesticide formulation labels. Additionally, some pesticide active ingredients can be considered PFAS because their structures contain at least one fully fluorinated methyl or methylene carbon atom (Organisation for Economic Co-operation and Development 2021 revised definition). To better understand the potential co-occurrence of pesticides and PFAS in predominantly agricultural watersheds, 10 streams were selected for sampling in northern California, USA. Agricultural inputs included rice, nuts (almonds, walnuts), tomatoes, alfalfa, and various row crops. Water samples were collected in May and July of 2024 and corresponded with peak target pesticide application timings. Water samples were analyzed for more than 180 pesticides and pesticide transformation products and 64 PFAS compounds. To evaluate potential PFAS occurrence associated with agricultural seed treatments, several types of insecticide and fungicide coated seeds (wheat, canola, soybeans, maize) were extracted and analyzed for more than 50 PFAS. This work will (1) compare the occurrence and concentrations of target pesticides (e.g. the insecticide methoxyfenozide) with commonly detected PFAS (e.g., perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate [PFOS]), (2) evaluate the contribution of pesticides that qualify as PFAS (e.g., the fungicide fluopyram) to overall PFAS concentrations, and (3) determine if PFAS are present in pesticide-coated seeds.

3.15.T-02 Co-Occurrence of Heavy Metals (Cu, Zn, Mg) and Pesticides in European Agricultural Soils: A European Assessment

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Modern agriculture has led to an increasing occurrence and levels of contaminants in soil, which may pose significant risks to environmental and human health. Over the years, an increasing number of studies worldwide have reported data on different soil contaminants, such as pesticides, heavy metals, microplastics, and pharmaceutical products. However, knowledge on effective soil contamination status remains incomplete/fragmented, also because most of the assessments focus on a single type of contaminants. Notably, there is a significant knowledge gap regarding contaminants co-occurrence and on its implications to soil health. The co-occurrence of pesticides and heavy metals is highly likely in agricultural soils and particularly concerning due to their intrinsic relationship, as pesticides contribute to heavy metal accumulation, and as copper based fungicides have a significant expression on conventional and organic farms. Soil serves as a primary sink and key reservoir for these contaminants, and the application of pesticides, particularly copper (Cu)-based fungicides, contributes to Cu being one of the most common heavy metals in agricultural soils.

This study, part of the H2020 project SPRINT, addresses the critical knowledge gap regarding the co-occurrence of contaminants in agricultural soils by assessing the occurrence and levels of multiple pesticide residues and of selected heavy metals (Cu, Zn, and Mn) across organic and conventional farming systems in Europe.

3.15.T-03 Continuous River Monitoring Networks: Understanding Co-Occurrence of Chemical Pollution from Urban and Rural Sources

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Water quality and freshwater biodiversity are declining globally due to complex mixtures of chemical pollutants from diverse sources. In England, only 16% of surface water bodies achieve good ecological status under the EU Water Framework Directive. To mitigate the impacts of chemical pollutants on UK freshwater biodiversity, a high-resolution monitoring study is needed that accounts for all chemical sources, prioritizes high-risk substances, and provides spatial and temporal insights into mixture profile variations within catchments. The ECOMIX project conducted a comprehensive high-resolution spatial and temporal monitoring study across 19 sampling sites in 9 English catchments to assess chemical contamination dynamics and evaluate chemical mixture impacts on freshwater biodiversity. Weekly composite samples collected over 12 months (March 2024-March 2025) were analyzed for 53 prioritized organic chemicals covering seven classes, selected based on ecotoxicity, bioavailability, and stakeholder input. Nineteen monitoring sites were selected using k-means cluster analysis based on topography, contaminant sources, land uses, and pathways. Preliminary analysis of the first two months revealed significant spatial and temporal variations in chemical concentrations. Urban sites demonstrated higher pollution levels compared to rural areas, with mean cumulative concentrations of 2763 ng/L and 1394 ng/L, respectively. Temporal variation was observed across all 19 sites, with the ratio of maximum to minimum total concentrations over the two-month period ranging from 1.5 at a location on the River Foss to 19.5 for a location on the River Wharfe. Downstream sections consistently displayed higher concentrations than upstream areas, particularly in rivers intersecting urban zones, driven by industrial effluents, urban runoff, and municipal wastewater discharge. Pharmaceuticals, pesticides, and personal care products were the dominant contaminants, with mean concentrations of Metformin (933 ng/L), Cypermethrin (157 ng/L), and Methylchloroisothiazolinone (117 ng/L) as key contributors in each class. This monitoring study has generated a large and systematic dataset, revealing spatial and temporal patterns of chemical contamination in urban and rural environments. The findings support the development of policy-driven strategies for effective pollution management and the mitigation of threats to freshwater biodiversity.

3.15.T-04 Size-Segregated Analysis of Airborne Nano- and Micro-Plastics and their Association with Carbonaceous Species in an Urban Environment

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The presence of nano- and micro-plastics (NMPs) in the air has emerged as a significant environmental and health concern. Airborne NMPs have been detected in outdoor environments, but most research is focused on the concentration of deposited particles, while submicron-suspended NMP particles, which directly impact human exposure, are understudied. Extensive studies exploring the interrelationships among polymers and their interactions with other urban pollutants, such as carbonaceous species, are lacking. Initiatives are needed to publish data on the extent of NMP pollution. Hence, the current study fills the gap in data scarcity to understand the dynamics, sources, and degradation of NMP pollutants as PM₁₀ NMPs, coarse microplastics (CMPs) as PM_{10-2.5} and fine microplastics (FMPs) as PM_{2.5} and their interaction with other carbonaceous parameters. We analysed synthetic polystyrene (PS), polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polyvinyl chloride (PVC), poly(methyl methacrylate) (PMMA), polycarbonate (PC), and TWPs in PM₁₀ and PM_{2.5} samples using pyrolysis-gas chromatography coupled with mass spectrometry (Py-GC-MS). The average total atmospheric NMPs concentration was found to be 0.6±0.1 µg/m³, 0.3±0.1 µg/m³ and 0.3±0.1 µg/m³ in PM₁₀ NMPs, FMPs and CMPs respectively. TWPs contribute ~60% to the NMPs composition in all size fractions. TTT, PMMA, PVC, and PC mostly contribute to FMPs, and PP, PE, PET, and PS contribute

more to CMPs. Airborne NMPs exhibit significant interactions with carbonaceous species, influencing their environmental fate and transport. In CMPs, primary organic carbon (POC) and elemental carbon (EC) show direct emission associations with NMPs, whereas correlation with secondary organic carbon (SOC) indicates their secondary formation during the degradation process in the atmospheric environment, especially in FMPs. The current NMPs quantification method is reliable, which includes a large spectrum of analysed polymers and assesses the relation of analysed NMPs with the carbonaceous species to understand their dynamics and environmental behaviour.

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3.15.P-Th240 Modelling Chemical Mixtures for Assessing the Impact on Freshwater Biodiversity in Yorkshire, UK

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Water quality and freshwater biodiversity are declining due to human-made stressors resulting from a mixture of chemicals from a wide range of sources. Nevertheless, studies often focus on a small number of chemicals from single source types, ignoring the fact that freshwaters are exposed to complex mixtures of hundreds of chemicals originating from multiple sources that interact together to exacerbate impacts. To move forwards, a more integrated approach is required that considers all significant sources of chemicals, focuses on those chemicals that pose the greatest risk, and delivers a spatial and temporal understanding of how mixture profiles vary in the catchment. The ECOMIX project aims to develop innovative approaches for chemical mixture exposure and effects monitoring and modelling using nine catchments in Yorkshire, England as its test system. A stakeholder workshop prioritised 40 chemicals, including pharmaceuticals, pesticides, metals, personal care products, PAHs, and livestock and pet medicines. Source density maps were developed based on spatial data for population, combined sewer overflows, crops, livestock, pet ownership, mines and road emissions, and these were combined with an analysis of dilution potential within the catchment. Density maps of the sources of chemicals were calculated for 50 sub-catchments within the study area. Subbasins with high source density areas are widespread within the catchment. A catchment-scale model was developed to consider inputs from multiple sources, land uses, and pathways. The model was used to generate current (2002-2022) profiles for flow and chemical exposure. Spatially distributed stream flow and nutrients were generated using the terrestrial hydrological and biogeochemical model HYPE for 16,000 separate river reaches. The model runs on a daily time step to capture individual storms. Modelled WWTP-treated effluent concentrations of down-the-drain chemicals were simulated within the same order of magnitude to observed concentrations. Work is ongoing to model exposure concentrations that will provide data for ecological modelling. This presentation provides an overview of the modelling approach, including results of chemical emissions and river concentration of chemical mixtures in the catchment.

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3.15.P-Th242 ENCORE: A Probabilistic Framework for ENvironmental CO-exposure and Risk Estimation

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The EU's Chemicals Strategy for Sustainability (CSS) aims to incorporate "combined exposure" risks, stemming from unintentional chemical mixtures in the environment, into regulatory risk assessments. Until a suitable predictive tool is available to identify mixtures of concern, it has been suggested to lower all safety limits for single chemicals by the so-called Mixture Allocation Factor (MAF). The general objective of the recently established project ENCORE (ENvironmental CO-exposure and Risk Estimation) is to develop a probabilistic modelling framework that allows to identify the priority chemicals for co-exposure risk at the watershed level.

In modelling, uncertainty (i.e. lack/incompleteness of data) and variability (i.e. known spread on data) is unavoidable, in areas such as usage information, model parameters and processes, monitoring data, and more. Considering environmental risk, there is also uncertainty on the hazard assessment. In ENCORE, a

probabilistic framework will be developed to characterise and propagate the uncertainty throughout the whole risk assessment model. In addition to uncertainty characterisation (allowing for probabilistic risk characterisation), the ENCORE framework allows to combine different sources of information (e.g. monitoring and modelling). More specifically, ENCORE will combine prior probabilities from modeled data with new evidence from monitoring data using Bayesian networks (BN), enhancing robustness of model predictions.

The ENCORE framework builds on an environmental fate model originally developed for large-scale European domains, but adaptable to specific regions, such as the Rhine and Danube river basins. One key part of the ENCORE project is to improve the accuracy of model predictions for specific watersheds through improving the quality of model input data, by integrating chemical use and emission information, tonnage data over time and local monitoring data, in combination with verified hazard data. Ultimately, ENCORE aims to prioritize high-risk co-exposure chemicals by accounting for spatial and temporal exposure patterns across large regions of Europe.

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3.15.P-Th243 An Indicator for Toxic Pressure of Mixtures in the Dutch Pesticide Atlas

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When the concentrations of chemical substances in the water are lower than the protective standard, it is assumed that this has no unacceptable effects. However, when there are mixtures of substances the risk is potentially increased and an additional assessment step may be performed to determine the combined toxic pressure. Toxic pressure offers a nuanced insight into the extent to which substances and mixtures affect water quality and aquatic life.

A study was conducted to introduce an indicator for (mixture) toxic pressure into the Dutch Pesticide Atlas. This interactive online atlas provides a national picture of pesticides in surface water based on monitoring data from regional and national water managers and from drinking water companies.

The indicator calculates the mixture toxic pressure as the fraction of aquatic species potentially affected by multiple substances (msPAF) using Species Sensitivity Distributions (SSDs). A set of indicators for mixture toxic pressure was developed and built into the Dutch Pesticide Atlas, which is available online for experts and the general public. The atlas contains different indicators that can be used to display and explore spatial and temporal trends in toxic pressure at the national, regional or local scale. There are also indicators to assess the absolute and relative contribution to this toxic pressure by individual pesticides present.

The toxic pressure that is calculated may be an underestimation when pesticides are present below their limit of quantification or when insufficient toxicity data are available to construct SSDs for particular compounds.

The indicators are illustrated with examples on the risk of mixtures. With the indicator and utilities it could be visualised, among others, that the number of sampling stations in the Netherlands with high and very high toxic pressure decreases over time.

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3.15.P Co-Occurrence of Contaminants in Urban and Rural Environments

3.15.P-Th232 Simultaneous Detection of Nanoplastics and Adsorbed Pesticides by Surface-Enhanced Raman Spectroscopy

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One of the most critical characteristics of nanoplastics is their ability to interact with other pollutants in the environment, particularly in water bodies, where they are widely found. This property has raised concerns about their potential to amplify the toxicity of associated pollutants, creating complex and challenging-to-monitor mixtures. A notable example is the interaction between nanoplastics and pesticides, which represent a group of chemical compounds with highly toxic potential widely used in

agriculture. In addition to their impact on aquatic organisms, pesticides are known for their harmful effects on human health, such as endocrine-disrupting, carcinogenic, and neurotoxic effects. Diquat is a highly toxic herbicide for aquatic organisms, as it can cause from cellular damage to death at relatively low doses. In this way, the toxic impact of both nanoplastics and pesticide can be exacerbated as they interact, as the presence of nanoplastics may prolong exposure to the pesticide or increase its concentration in specific organisms. Despite the relevance of this interaction, studies on the detection of pesticides adsorbed on nanoplastics have not been reported, mainly due to the analytical challenges involved. In this context, the present study aimed to investigate the use of the Surface Enhanced Raman Spectroscopy (SERS) technique, employing gold nanoparticles as a substrate, to detect polystyrene (PS) nanoplastics (100 nm) and the pesticide Diquat in environmental relevant concentrations (as low as 20 µg/L for the polymer and 66 µg/L for the pesticide). By utilizing the SERS technique, it was possible to obtain the spectra of both compounds simultaneously, demonstrating the method's sensibility to detect both the nanoplastic and the adsorbed pesticide at very low concentrations. The results indicate that SERS is a promising tool for environmental analyses, providing a non-destructive, sensitive and specific alternative for the detection of nanoplastics and pesticides. The simultaneous acquisition of nanoPS and Diquat spectra opens up new possibilities for monitoring these pollutants in the environment and investigating the consequences of their interaction in different environmental scenarios.

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3.15.P-Th233 Synergistic Effects of Microplastics and Chemical Residues: Disruption of Soil Microbial Communities

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Soil is one of the most biologically diverse environments on earth. Soil microorganisms play a key role in driving soil functions, such as the cycling of carbon and nutrients, and thereby contribute to important ecosystem services. However, human activities result in various pollutants entering the soil, which are potentially hazardous to the soil ecosystem. In recent years, studies have typically focused on the toxicity of these pollutants for soil microbial communities by examining each of these compounds individually or limited to a mixture of one group (e.g. pharmaceuticals). However, these compounds are part of more complex mixtures of chemical pollutants and biological hazards. Moreover, the presence of nonbiodegradable particles in the soil matrix such as microplastics may substantially influence the toxicity and persistence of other chemicals in the soil. Diclofenac, terbutryn, ciprofloxacin, 17 α -ethynylestradiol (EE2) and diuron were selected for this study and applied as a mixture. These contaminants belong to various groups with distinct chemical structures and physicochemical properties, and are included in respective EU legislations of substances that should be monitored in the environment and the food chain. Polypropylene microplastics (PP-MPs) were selected as a background contaminant applied on its own or in a mixture with the other selected chemicals. PP-MPs are highly resistant to biodegradation due to their robust molecular structure. The presence of PP-MPs with the other chemicals in the tested soil substantially elevated the activity of the soil microbial community as reflected by increased respiration, from 0.40 0.70 µg CO₂-C g⁻¹ h⁻¹ in the control, PP-MPs, and mixture of tested chemicals separately, compared to 2.0 µg CO₂-C g⁻¹ h⁻¹ in combination (p = 0.009 - 0.02). The results demonstrated synergistic effects of chemical pollutants and microplastics on the soil microbial community. These findings underscore the complex interplay between chemical residuals and microplastics particles in natural soil, and the need for further research to understand the combined impacts and long-term implications of these pollutants and non-biodegradable particles on soil health and carbon cycling. This provides an important input for accurate and comprehensive environmental risk assessment of chemical contamination in the terrestrial environment.

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3.15.P-Th234 Sorption and Desorption Processes of S-Metolachlor in Soil Contaminated with Microplastics

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The increasing use of plastics in agriculture has resulted in the accumulation of microplastics (MPs), which can interact with residual herbicides and alter their environmental behavior. This study aimed to evaluate the influence of different MP proportions on the sorption and desorption processes of S-metolachlor in soil. The herbicide (1200 g a.i. ha⁻¹) was applied in soil at MPs concentrations of 0% (control unamended soil), 1, 5, 10, 15, 20, and 100% (w w⁻¹) MPs, with the addition of 2 g of material (soil or MPs). Sorption and desorption isotherms were determined using the batch equilibrium method, followed by herbicide analysis via high-performance liquid chromatography (HPLC). The results indicated that the presence of MPs altered S-metolachlor sorption by approximately 10% in soil amended with 5% MPs, with K_d (sorption coefficient) values ranging from 3.20 to 4.85 L kg⁻¹ in unamended soil, suggesting increased herbicide retention in the presence of MPs. The K_d (desorption coefficient) ranged from 3.20 to 33.20 L kg⁻¹, indicating that desorption increases with the MPs proportion, suggesting that S-metolachlor is more firmly retained in the MPs than in the soil. These findings suggest that the presence of MPs in soil can modify the environmental fate of S-metolachlor, potentially affecting their efficacy in weed control and increasing the risk of environmental contamination, particularly in agricultural soils polluted with these polymers.

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3.15.P-Th235 Exploring the Potential of Earthworms to Accelerate the Degradation of Biodegradable Plastics in Soils

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Plastic products have long been used in agricultural production and the release of plastic additives and plastic fragments has recently caught attention, sparking a trend towards environmentally friendly alternatives. As a consequence, plastics derived from biodegradable polymers have been actively promoted in the market. However, whether the use of biodegradable plastics (BPs) can really solve this problem remains a question. Our previous studies showed that BPs made from polylactic acid (PLA), polybutylene adipate terephthalate (PBAT) and starch can temporarily affect the activity, composition and functional diversity of soil microbial communities, which was synchronized with BPs degradation. Another major finding was that BPs made from PLA and PBAT degraded very slowly in certain soils, which can lead to the accumulation of BPs fragments in soils. Therefore, given the uncertain environmental impacts of BPs and their unsatisfying degradation rate, efforts were made to accelerate the degradation of BPs in soils. The potential of earthworms to reduce BPs in soils was explored under controlled conditions in the lab. *Lumbricus terrestris* was exposed to soils spiked with microplastics (MPs) of different polymer types at a dosage of 1% (dw/dw). The changes of MPs during gut passage and cast aging were investigated. The size distribution of MPs in different niches (soil, gut, cast) was determined by a laser direct infrared (LDIR) chemical imaging system. Recovered MPs (either in the form of MPs by density separation or in the form of polymers by Soxhlet extraction) were characterized by different techniques, e.g., FTIR, GPC, DSC and NMR. Results show that *Lumbricus terrestris* was able to initiate the physical fragmentation of MPs in their gut. Certain BPs (PLA) can be depolymerized to some extent in the gut. After being excreted in the cast, low density polyethylene did not show any changes during cast aging. PLA degradation was accelerated in the cast than in the soil, accompanied by the reduction in particle size. PBAT MPs were fragmented in the gut but no further degradation happened during cast aging. Our studies show that earthworms do have the potential to accelerate the degradation of certain plastics, especially less recalcitrant ones. However, the simultaneous fragmentation of plastics into smaller pieces by earthworms might lead to an input smaller MPs back to soils. The fate of MPs after earthworm gut processed needs further study in the future.

3.15.P-Th236 Levels, Spatial Distributions, and Provision of Petroleum Hydrocarbons and Phthalates in Sediments from Obhur Lagoon, Red Sea Coast of Saudi Arabia

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The levels, spatial distribution, and sources of petroleum hydrocarbons and phthalates were assessed in surface sediment samples from the urban lagoon of Obhur near Jeddah, the largest city on the Red Sea Coast of Saudi Arabia. The lagoon was divided into the inner zone (IZ), middle zone (MZ), and outer zone (OZ) based on its geomorphological features and developmental activities. Surface sediment samples were collected and extracted with a mixture of dichloromethane:methanol and total extractable organic matter (TEOM) of each sample was analyzed with gas chromatography-mass spectrometry (GC-MS). n-Alkanes, hopane and sterane biomarkers, and unresolved complex mixture (UCM) were the major petroleum hydrocarbon compounds of the TEOM. Phthalates were also measured in the sediment samples. In the three zones, n-alkanes ranged from 89.3 ± 88.5 ng/g to 103.2 ± 114.9 ng/g, whereas the hopane and sterane biomarkers varied from 69.4 ± 75.3 ng/g to 77.7 ± 69.9 ng/g and 72.5 ± 77.9 ng/g to 89.5 ± 82.2 ng/g, respectively. The UCM concentrations ranged from 821 ± 1119 ng/g to 1297 ± 1684 ng/g and phthalates from 37.4 ± 34.5 ng/g to 65 ± 68 ng/g. The primary origins of these anthropogenic hydrocarbons in the lagoon sediments were petroleum products (boat-engine discharges, boat washing, lubricants, and wastewater flows) and plasticizers (plastic waste and litter). The proportions of anthropogenic hydrocarbons derived from petroleum products in the sediment's TEOM ranged from $43 \pm 33\%$ to $62 \pm 15\%$, while the percentages for plasticizers varied from $2.9 \pm 1.2\%$ to $4.0 \pm 1.6\%$. The environmental impacts of petroleum and phthalate in coastal sediments are profound and multifaceted. Their persistence, toxicity and ability to bioaccumulate make them critical pollutants of concern for marine ecosystems. Their occurrence in sediments leads to chronic exposure for marine species and potential bioaccumulation across the food web. Addressing these impacts requires a multifaceted approach, including stricter requirements, pollution control measures, and ongoing monitoring to protect and restore coastal health. Understanding these pollutants behavior and effects in marine environments is essential for developing effective strategies to mitigate their negative influence on coastal and marine ecosystems.

3.15.P-Th237 Setting up a National Monitoring Program to Identify Ubiquitous and Potential Harmful Substances in Soil and Sediment in the Netherlands

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Non-standardized substances are substances that currently lack established legal standards regarding permitted concentrations in the environment. The absence of such standards complicates efforts for regulatory authorities and businesses to provide guidelines for the safe use and management of these substances and/or the materials in which they may be present (such as soil and sediment). A well-known example of this challenge is the current PFAS crisis.

Therefore, the pilot Monitoring for Non-standardized Substances (pMNS) was set up in early 2024 with the aim to develop a novel national approach for detecting various non-standardized substances that co-occur and are diffusely spread across both soil and sediment in the Netherlands and may pose environmental risks. To achieve this, 440 soil and 280 sediment samples with different land use types (including urban and rural) are being collected from various locations across the Netherlands. A part of each sample is being analyzed for initially five groups of substances: polychlorinated n-alkanes (also known as chlorinated paraffins), polybrominated diphenyl ethers, glyphosate and its transformation product AMPA, pyrethroids and PFAS precursors. The selection of the five groups of substances was based on their persistence, toxicity, bioaccumulation and emission potential. Variability among the substance groups, including differences in their use and chemical properties, was also taken into account. The rest of the sample is stored under controlled conditions for future analysis of other substances and other purposes. Indicative risk thresholds for health and background levels will be derived for the substance groups that are found to be present, which can be used by regulatory authorities. This poster presents the aim, methodology, challenges, preliminary results and potential next steps of the pilot project.

3.15.P-Th238 A 600-Years Pollution History Reconstruction Using Lake Sediments from Bad Waldsee, Southern Germany

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Annual lake sediments are excellent high-resolution archives for reconstructing historical pollution. Usually, pollution reconstructions date back to the beginning of industrialization in the early 19th century. Here, we present a long-term record from Lake Stadtsee located in the city of Bad Waldsee. Using historical written documents, the history of pollution can be linked to the economic activities in the city. In an absolutely dated sediment core we explored the concentration and composition patterns of Polycyclic Aromatic Hydrocarbons (PAHs) and heavy metals together with macro charcoal record at 1 cm steps. First findings indicate that PAHs were primarily derived from pyrogenic, rather than petrogenic sources throughout the entire time interval. These most probably correspond to urban fire or domestic heating. The general trend observed by using PAH diagnostic ratios indicates a shift of the sources from low temperature pre-industrial combustion processes towards high temperature combustion processes in more recent times. Macro charcoal analysis revealed two main phases of biomass burning, which were separated by a fire free interval for more than 200 years. The first phase in the late Medieval period (653?533 cal BP), shows high proportions of burned grass and monocot leaves, whereas in the second phase in the early Modern Times (313 cal BP until today) wood was the main fire fuel. The molecular ratio pattern of specific methylated-phenanthrene isomers also indicates a change of fuel sources. Heavy metal analyses revealed an increasing Pb sediment concentration over time (more than 2x), while V, Ni, Cu and Zn were present in the same concentration order. Interestingly, during the 30-year war all heavy metals analyzed showed a remarkable decrease in concentration, which returned to the previous level after the war.

3.15.P-Th239 Determining the Impact of Pyrolytic Conversion on the Exposure and Mobility of Organic Pollutants, Metals and Nutrients in Biochar

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The Danish Government has presently a strong focus on pyrolysis as part of the green transition in Denmark. This is due to its potential and increasing applications within circular economy, carbon sequestration, soil improvement, and contaminant removal. However, while organic contaminants present in the feedstock can be broken down during pyrolysis, new contaminants, like PAHs, can be formed, and metals can be concentrated in the produced biochar. Consequently, the agricultural application of biochar derived from waste materials such as sewage sludge could potentially contaminate our environment. This raises the critical question: How does the pyrolytic conversion of feedstock to biochar affect the exposure, mobility and potential impact of pollutants in the environment? The first aim of this study is to determine the combined impact of the pyrolytic conversion on (1) the pollutant concentrations in the matrix and (2) the corresponding distribution coefficients. Digested sludge from WWTPs is used as model feedstock, with carefully matched samples of sludge and biochar obtained from a full-scale pyrolysis plant in Denmark. Aqueous concentrations in equilibrium with sludge and biochar are then measured across a broad range of organic chemicals, metals and nutrients, utilizing advanced analytical techniques such as LC-MS and SPME-GC-MS. We hypothesize that pyrolysis markedly decreases aqueous concentrations for most if not all pollutants. Special focus is given to extending this work to non-targeted chemical analysis to comprehensively assess the impact of pyrolytic conversion on various pollutants, including PFAS. The outcomes of this study will be used to provide further information to the Danish EPA on the potential environmental impact of biochar application in agriculture.

3.15.P-Th240 Modelling Chemical Mixtures for Assessing the Impact on Freshwater Biodiversity in Yorkshire, UK

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Water quality and freshwater biodiversity are declining due to human-made stressors resulting from a mixture of chemicals from a wide range of sources. Nevertheless, studies often focus on a small number of chemicals from single source types, ignoring the fact that freshwaters are exposed to complex mixtures of hundreds of chemicals originating from multiple sources that interact together to exacerbate impacts. To move forwards, a more integrated approach is required that considers all significant sources of chemicals, focuses on those chemicals that pose the greatest risk, and delivers a spatial and temporal understanding of how mixture profiles vary in the catchment. The ECOMIX project aims to develop innovative approaches for chemical mixture exposure and effects monitoring and modelling using nine catchments in Yorkshire, England as its test system. A stakeholder workshop prioritised 40 chemicals, including pharmaceuticals,

pesticides, metals, personal care products, PAHs, and livestock and pet medicines. Source density maps were developed based on spatial data for population, combined sewer overflows, crops, livestock, pet ownership, mines and road emissions, and these were combined with an analysis of dilution potential within the catchment. Density maps of the sources of chemicals were calculated for 50 sub-catchments within the study area. Subbasins with high source density areas are widespread within the catchment. A catchment-scale model was developed to consider inputs from multiple sources, land uses, and pathways. The model was used to generate current (2002-2022) profiles for flow and chemical exposure. Spatially distributed stream flow and nutrients were generated using the terrestrial hydrological and biogeochemical model HYPE for 16,000 separate river reaches. The model runs on a daily time step to capture individual storms. Modelled WWTP-treated effluent concentrations of down-the-drain chemicals were simulated within the same order of magnitude to observed concentrations. Work is ongoing to model exposure concentrations that will provide data for ecological modelling. This presentation provides an overview of the modelling approach, including results of chemical emissions and river concentration of chemical mixtures in the catchment.

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3.15.P-Th241 Assessing Emerging Contaminants and Biodegradation Potential in the Atlantis Aquifer's Managed Aquifer Recharge Scheme

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Managed aquifer recharge (MAR) using treated effluent and stormwater (TES) is an important strategy for sustaining urban aquifers used for potable water supply in water-scarce regions. However, there are concerns about the introduction of emerging contaminants (ECs) present in TES. This study aims to assess the presence and spatial distribution of various multi-group ECs including pharmaceuticals, pesticides and industrial chemicals in the Atlantis Aquifer. Twenty samples, including MAR source water, groundwater, and surface water, were analysed for 289 compounds. Preliminary results show that 120 compounds were detected, with TES having the highest number of detections, followed by MAR-impacted groundwater, while naturally recharged groundwater had the lowest number of detections. The results suggest that ECs are introduced to the aquifer through MAR, that some ECs may be attenuated, and that there is a baseline level of contamination in naturally recharged groundwater, likely from atmospheric deposition and agricultural runoff. A three-tiered health risk assessment revealed that fewer than 10% of detected compounds pose health risks ($RQ > 1$). Further investigations will consider the behaviour and health risks of co-occurring compounds. To assess natural attenuation via biodegradation in the aquifer, traditional contaminants and parameters (e.g., redox, nitrate, dissolved oxygen, manganese) will be analysed using data collected between 2018 and 2024 from 253 sites. The outcomes of the study will guide the development of an EC monitoring framework for the Atlantis Aquifer water supply scheme, which can also be adapted to South Africa's other MAR schemes in development, as well as other schemes across Africa.

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3.15.P-Th242 ENCORE: A Probabilistic Framework for ENvironmental CO-exposure and Risk Estimation

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The EU's Chemicals Strategy for Sustainability (CSS) aims to incorporate "combined exposure" risks, stemming from unintentional chemical mixtures in the environment, into regulatory risk assessments. Until a suitable predictive tool is available to identify mixtures of concern, it has been suggested to lower all safety limits for single chemicals by the so-called Mixture Allocation Factor (MAF). The general objective of the recently established project ENCORE (ENvironmental CO-exposure and Risk Estimation) is to develop a probabilistic modelling framework that allows to identify the priority chemicals for co-exposure risk at the watershed level.

In modelling, uncertainty (i.e. lack/incompleteness of data) and variability (i.e. known spread on data) is unavoidable, in areas such as usage information, model parameters and processes, monitoring data, and

more. Considering environmental risk, there is also uncertainty on the hazard assessment. In ENCORE, a probabilistic framework will be developed to characterise and propagate the uncertainty throughout the whole risk assessment model. In addition to uncertainty characterisation (allowing for probabilistic risk characterisation), the ENCORE framework allows to combine different sources of information (e.g. monitoring and modelling). More specifically, ENCORE will combine prior probabilities from modeled data with new evidence from monitoring data using Bayesian networks (BN), enhancing robustness of model predictions.

The ENCORE framework builds on an environmental fate model originally developed for large-scale European domains, but adaptable to specific regions, such as the Rhine and Danube river basins. One key part of the ENCORE project is to improve the accuracy of model predictions for specific watersheds through improving the quality of model input data, by integrating chemical use and emission information, tonnage data over time and local monitoring data, in combination with verified hazard data. Ultimately, ENCORE aims to prioritize high-risk co-exposure chemicals by accounting for spatial and temporal exposure patterns across large regions of Europe.

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3.15.P-Th243 An Indicator for Toxic Pressure of Mixtures in the Dutch Pesticide Atlas

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When the concentrations of chemical substances in the water are lower than the protective standard, it is assumed that this has no unacceptable effects. However, when there are mixtures of substances the risk is potentially increased and an additional assessment step may be performed to determine the combined toxic pressure. Toxic pressure offers a nuanced insight into the extent to which substances and mixtures affect water quality and aquatic life. A study was conducted to introduce an indicator for (mixture) toxic pressure into the Dutch Pesticide Atlas. This interactive online atlas provides a national picture of pesticides in surface water based on monitoring data from regional and national water managers and from drinking water companies.

The indicator calculates the mixture toxic pressure as the fraction of aquatic species potentially affected by multiple substances (msPAF) using Species Sensitivity Distributions (SSDs). A set of indicators for mixture toxic pressure was developed and build into the Dutch Pesticide Atlas, which is available online for experts and the general public. The atlas contains different indicators that can be used to display and explore spatial and temporal trends in toxic pressure at the national, regional or local scale. There are also indicators to assess the absolute and relative contribution to this toxic pressure by individual pesticides present.

The toxic pressure that is calculated may be an underestimation when pesticides are present below their limit of quantification or when insufficient toxicity data are available to construct SSD s for particular compounds.

The indicators are illustrated with examples on the risk of mixtures. With the indicator and utilities it could be visualised, among others, that the number of sampling stations in the Netherlands with high and very high toxic pressure decreases over time

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3.15.P-Th244 How PFAS End up in High Concentrations in Home Produced Chicken Eggs at Ambient Background Levels in Soils

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In an area downwind from a fluorochemical plant in the Netherlands, high concentrations of PFAS have been found in home produced chicken eggs. The concentrations of PFOS and PFOA exceed the Maximum Level for eggs (EU, 2022) in 80% of the eggs. This is in contrast to the level of PFAS found in commercial eggs, which is very low. Although the eggs are sampled in an area surrounding a fluorochemical plant, the detection of high amounts of PFOS is remarkable, since the plant predominantly emitted PFOA and GenX. PFOS is the dominant PFAS detected, whereas this compound has not been used by the fluorochemical plant in their production process. Furthermore, PFOS concentrations in the soil are at approximately the same level as ambient background levels in the Netherlands in urban areas. Based

on the results it was concluded that direct consumption of soil by the egg-laying chickens was not the source of the high concentrations of those PFAS found in the eggs.

This resulted in a quest for finding the source of these high concentrations of PFAS. At a selection of the sites, the chicken housing was sampled for possible PFAS sources - feed (including insects), soil (near the feeding unit), drinking water, packaging of the feed, bedding, vitamins, medicines and worms.

The PFAS, especially PFOS, concentrations detected in the worms were very high, in contrast to the other possible sources analyzed. This led to the conclusion that bioaccumulation of PFAS via worms is the most likely source of PFAS for the chicken and the eggs, even at the relatively low concentrations of PFAS measured in the soils.

In the presentation we would like to show the results and put them in the perspective of (background) concentrations measured in soils and eggs on a national level.

The risk of consuming the eggs was evaluated by RIVM (National Institute for Public Health and the Environment). They gave a negative consumption advice for the eggs in the area. RIVM is now working on a national inventory on PFAS in home produced chicken eggs.

The project was conducted for the local municipalities (Dordrecht, Papendrecht, Sliedrecht, Molenlanden), by Arcadis, RIVM, WFSR (Wageningen Food Safety Research) and Tritium Advies, in cooperation with the OZHZ (Environment Agency South-Holland South) and GGD ZHZ (Public Health Service South-Holland South).

3.15.P-Th245 Co-Occurrence Patterns of Semi-Volatile Organic Compounds in Urban and Rural School Environments in the Czech Republic

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Exposure to SVOCs in schools has gained growing attention from researchers and the public. Many schools were built when legacy SVOCs were used as additives in building materials. With some SVOCs now banned, it is hypothesized that newer or recently renovated schools will have lower concentrations of these legacy compounds. Here, we evaluated ten schools of different ages located in urban and rural areas in a single region in the Czech Republic to compare levels of SVOCs in indoor and outdoor air and indoor dust. Legacy industrial compounds (e.g., polychlorinated biphenyls PCBs; polybrominated diphenyl ethers PBDEs) and organochlorine pesticides (OCPs) were detected in 60 to 100% in air and dust samples, indicating their widespread presence in school environments. Dechlorane Plus and alternative bromobenzene flame retardants (bromobenzene) were also detected in 60 to 100% of all air and dust samples. BDE 209 dominated the PBDE distribution profile in air and dust samples, accounting for over 50% of the total PBDEs. γ -HCH (lindane) was the dominant OCP, with concentrations reaching up to 116 ng/g in indoor dust and 9.12 ng/m³ in indoor air. Concentrations in indoor air were dominated by flame retardants and PCB-11, while DDT and PCBs dominated in outdoor air. In both urban and rural locations, the indoor air concentrations were substantially higher than outdoor, e.g., 6 to 50 times higher for PCBs and 4-400 times higher for HCHs. This indicates that the SVOCs primarily originate from indoor sources, regardless of outdoor levels. Older, more urban schools had the highest concentrations of both DDTs and HCHs and moderately high PBDE concentrations in both air and dust. Less urban schools were generally newer buildings and showed lower OCP and PBDE levels. SVOC presence appears to be related to emissions from specific construction materials and the continued presence of hazardous SVOCs in schools raises concerns about children's potential exposure.

3.15.P-Th246 Soil Health and Food Quality in Horticultural Systems: A Case Study in the Metropolitan City of Naples

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Urban areas, covering over 25% of Europe's territory and hosting 75% of its population, a figure projected to rise to 80% by 2050, face pressing challenges, including urban sprawl, green space decline, and soil degradation. As a cornerstone of the urban ecosystem, soil supports biodiversity, provides ecosystem

services, and contributes to citizen well-being. In the last decades, the metropolitan city of Naples (a densely populated area with 3 million inhabitants) was interested in significant soil consumption and infrastructure sprawl. The urban soil used for residential and productive activities currently covers approximately 35% of the metropolitan area (ISPRA, 2023).

Although many of the remaining soils are used for farming and food production, only scant information is available on the fertility and health of these soils, and the quality/safety of their food products. The 2-year HealthySoil4QualityFood project (CUP-E53D23011040006, financed in the frame of European Union Next Generation EU), intends to encompass this gap, with research activities aimed at providing insight on the: i) soil fertility and possible chemical contamination in vegetable gardens of Naples metropolitan area; ii) irrigation water quality; iii) crop biodiversity and sustainability of cultivation practices, and chemical quality of edible biomasses; iv) risks associated to possible contamination, throughout integrated environmental and human exposure modelling; v) public awareness about soil ecosystem services and social benefits of urban farming.

Five case studies were recently selected based on geographical distribution and proximity to potential sources of contamination, type of stakeholders, and scope of cultivation (arable soils split in plots and assigned to citizens, or green spaces used as educational gardens for school, etc); in these areas, sited in the districts of Capodimonte, Ponticelli and Vomero of Naples and in the municipality of Portici, we started assessing the soil physicochemical properties and fertility and irrigation water quality. The first results evidenced an overall good quality of urban soils, showing a sandy-loam texture, neutral-to-sub-alkaline pHs, good nutrient bioavailability, and low-to-moderate occurrence/bioavailability of contaminants. Food products' nutritional value and safety will be monitored in different growing seasons. Realistic exposure scenarios to possible contaminants in each case study will be developed by the MERLIN-Expo tool.

3.15.P-Th247 Aliphatic and Cyclic Hydrocarbons in Urban Street Dust from Riyadh City, Saudi Arabia: Levels, Distribution, and Sources

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Dust particles contain diverse natural and anthropogenic organic compounds and act as local collectors of pollutants, particularly in urban settings. Street dust samples were collected from various sites in Riyadh City, Saudi Arabia in 2023. These were extracted using dichloromethane methanol mixture, and the resulting extracts were subjected to analysis through gas chromatography mass spectrometry (GC MS). The primary compounds identified included n-alkanes, methyl n-alkanoates (FAMES), hopanes, steranes, polycyclic aromatic hydrocarbons (PAHs), plasticizers, tobacco miscellanies, and an unresolved complex mixture (UCM). Vegetation detritus constituted the primary natural source of organic compounds, ranging from 7.4±3.5% to 15.0±4.0%, and comprised fractional n-alkanes and FAMES. Petroleum-related products from vehicular emissions, and oil combustion were predominant, accounting for 73.3±5.1% to 87.5±4.8%, and included partial n-alkanes, hopanes, steranes, PAHs, and UCM. Littering inputs from discarded plastics and tobacco smoking varied from 5.2±1.3% to 12.0±5.3% and included phthalates, nicotine, and cotinine, as well as recreational drinking (coffee and tea beverages containing caffeine). The occurrence and distribution of natural and anthropogenic extractable organic matter in this arid urban area were influenced by local vegetation and human activities. The prevalence of anthropogenic organic compounds in Riyadh city's street dust depended on locations and types the location and type of urban activities, with elevated levels observed in high-traffic and industrial zones. Aliphatic and cyclic hydrocarbons are of significant concern due to their potential impact on public health, especially in urban areas with high levels of vehicular traffic and industrial activities, which contribute significantly to air and soil pollution. The health effects of exposure to these hydrocarbons can range from respiratory issues to carcinogenic risks, particularly for vulnerable populations such as children, elderly individuals, and those with pre-existing health conditions. Consequently, further investigations are necessary to understand the potential health effects of anthropogenic inputs on the city's residents.

3.15.P-Th248 Measuring and Modelling Deposition and Washoff of Particulate Matter in Tree Leaves in an Urban Forest Ecosystem Gradient

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Urban and rural forests are known to improve air quality by contributing to the removal of particulate matter (PM) from the air. The deposition rate of PM on tree leaves depends on several parameters, including particle size and concentration, plant species, leaf traits, and meteorological conditions. While many previous studies have investigated PM deposition and accumulation on leaves, there is still little information about removal mechanisms and dynamics, such as washoff. We selected Laurel (*Laurus nobilis* L.) for this study because it is found at many temperate latitudes and since it is an evergreen, it is ideal for investigating the long-term deposition and removal rates of PM. Here, we investigated PM deposition and washoff from its leaves with different chemical and imaging techniques. Leaves were collected at a control site in a relatively remote area along Lake Como and an urban site near a busy road in Como, Italy. Leaves were washed at different intensities (gently rinsed to simulate precipitation and sonicated to obtain the total PM deposited). The mass of unwashed, gently washed, and sonicated leaves were then determined gravimetrically to obtain the amounts of deposited PM and their release rates at different washing intensities. Leaves were then analyzed with an optical microscope, a scanning electron microscope (SEM), and a white light interferometer to study number, size, and spatial distribution of particles. The results showed the relationships among deposited and accumulated PM, PM air concentration (obtained from the Regional Environmental Protection Agency/ARPA Lombardia), rainfall intensity and amount, and washoff rates at different conditions and different pollution levels. The imaging techniques allowed us to gain insight into particle density, their size and count at different resolutions, further evidencing the role of plant leaves in aggregating small atmospheric PM and later, in part, returning them to the soil environment.

3.15.P-Th249 Sustainable Green Roofs for the Implementation of the Sponge City: Determination of Ecotoxicologically Safe Construction Products for Green Roofs *Elena Perabo and Christoph Hafner, Hydrotex - Labor für Ökotoxikologie und Gewässerschutz GmbH, Germany*

The consequences of climate change pose enormous challenges for urban regions. In addition to prolonged periods of heat, climate change also affects the availability of water in particular: both heavy rainfall events with flooding and droughts lead to significant damage to buildings, infrastructure and ecosystems, among other things. The sponge city concept offers various advantages for meeting these challenges: in addition to retaining rainwater, preventing flooding and relieving the burden on wastewater systems, it reduces the urban heat island effect through evaporative cooling and reduces drought.

To a large extent, this concept is to be implemented using green roofs, which utilize construction products that are regularly exposed to rainwater. These construction products may have negative impact on the retained water due to the leaching of hazardous substances, which are subsequently discharged into surface water and/ or groundwater. Thus the leaching of substances may cause ecotoxic and human-toxic effects. In the research project, building materials used for the construction of green roofs are eluted in leaching tests and the eluates are analyzed for potential negative effects on the aquatic environment using a standardized bioassay battery. Biotests offer an integrative approach to detecting the effects of undesirable substances, capturing the cumulative impact of complex mixtures, including unidentified or unexpected compounds. In contrast, analytical methods rely on a targeted approach, requiring prior knowledge of specific substances to be identified and quantified. In the further course, a model roof is constructed on a reduced scale with positively evaluated building products and the retained and discharged rainwater is examined with regard to its ecotoxic effects. In comparison, roof run-off water from practical projects will also be examined. The aim is to define building materials that can be used safely for the environment as part of the sponge city concept for green roofs.

The ecotoxicological assessment follows the CEN/TS 17459 framework, which combines standardized leaching tests with ecotoxicological evaluations. Depending on the product type, two different leaching methods are applied: for flat and monolithic products, the Dynamic Surface Leaching Test (DSL_T, as per DIN EN 16637-2) is used, while granular products are assessed with the upflow percolation test (DIN EN 16637-3).

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3.15.P-Th250 Road Runoff in Urban Areas: Monitoring and Evaluation of a Raingarden Through One Year of Sampling

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The negative impact of road pollution, especially tire wear particles (TWP), has gained attention due to new studies on their chemical content, leaching capabilities, and effects on organisms. This study investigated the seasonal variation of pollutants in road runoff, including TWP, organic compounds, metals, and water quality parameters, to assess the mitigation efficiency of a raingarden. Online sensors monitored the inlet and outlet of the raingarden continuously, providing data on water level, temperature, turbidity, and conductivity. Seven rainfall events were monitored: June (01), September (02, 03), October (04), November (05) 2023, and May (06), July (07) 2024. TWP was analysed PYR-GC/MS, organic compounds were analysed using HPLC-MS/MS and metals using ICP-MS. TWP levels varied between rainfalls but were higher at the inlet (2.57 ± 2.30 mg/L) compared to the outlet (0.488 ± 0.188 mg/L), resulting in an 81% reduction. For selected organic compounds (6PPD-Q, Benzothiazole (BT), Methylthiobenzothiazole (MTBT), Hydroxybenzothiazole (OHBT), Aminobenzothiazole (ABT), and Mercaptobenzothiazole (MBT)), levels varied between rainfall events, with the highest levels in Rainfall 01. The highest inlet level was for BT (9.73 ± 6.76 µg/L), followed by OHBT (9.29 ± 7.72 µg/L), MTBT (1.23 ± 1.08 µg/L), ABT (0.380 ± 0.247 µg/L), and 6PPD-Q (0.227 ± 0.160 µg/L). Comparing inlet to outlet values suggests retention rates of 90% for MTBT, 81% for 6PPD-Q, 71% for OHBT, 68% for BT, and 56% for ABT. For all rainfall events, the highest metal levels were for Na (77.8 mg/L), likely due to road salt (NaCl) entering the raingarden during winter. This is supported by high Cl levels (94.2 mg/L) and negative retention for both Cl (-248%) and Na (-215%), as well as high conductivity observed by the sensors. Another interesting result is the negative retention of Zn (-245%), with higher Zn levels in the outlet compared to the inlet. Zn is often used as an inorganic marker for TWP, but the high Zn levels in the outlet do not match TWP or organic tire-related compound levels. High correlation ($R > 0.8$) was found between 6PPD-Q and ABT (0.86), BT (0.94), and OHBT (0.86). No correlation was found between TWP or TSS and tire-related compounds. Further analysis, including sensor data, will be presented at the conference. This data is important for understanding the presence of these compounds in road runoff and assessing raingardens as urban nature-based solutions.

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3.15.P-Th251 Review of Three Years Environmental Pollutant Monitoring Along Railway Tracks in Germany

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In general, rail transport emits lower levels of pollutants than other modes of transport, but rail operations still have an impact on the environment. Inorganic and organic substance inputs come from rail operations, e.g. abrasion processes, and associated infrastructure maintenance work, such as vegetation control measures. The aim of the study is to summarize the results of three years pollutant monitoring and to categorize the dispersion of pollutants along the route into the adjacent environment. The results allow to close knowledge gaps and provide a basis for an environmental risk assessment.

Since 2021, the pollutant input from railway operations has been recorded at five long-term monitoring sites and their transport behavior was characterized. The investigation program includes the analysis of heavy metals, herbicides (incl. metabolites) and PAHs in water and soil samples. As part of the investigations, the depth-dependent herbicide and heavy metal contamination was determined in various soil horizons and track areas. In addition, the pollution potential at different distances from the track axis (2.5 m and 5.0 m) was also investigated. All sampling and analyses were carried out using standardized methods.

The results show a site-specific pollutant distribution with a clear decrease in pollutant concentrations from the middle of the track to its surroundings. The highest pollutant load was detected in the upper soil layers (0 - 0.30 m). Herbicides such as glyphosate and its metabolite AMPA, pelargonic acid and flumioxazine can be detected in the soil and leachate, especially after vegetation control. In a direct

comparison of samples from upstream and downstream groundwater in the track area, arsenic, iron and zinc concentrations were found to increase, with none of the values exceeding the regulated limits. The aim of the study is to combine, bundle and categorize the knowledge obtained to date and thus, improve our understanding of the potential release of pollutants caused by rail traffic. The results contribute to an initial risk assessment and a further sustainable development of rail transport.

3.16 Photochemical Transformation of Contaminants in Aquatic Environments

3.16.T-01 Following the Photochemical Fate of Fluorinated Compounds

William Arnold, *Thomas Mundhenke, Akash Bhat, Cole Scholtz and William Pomerantz, University of Minnesota, United States*

Rachel Carson brought to light the dangers of persistent chlorinated chemicals in 1962, and we have spent decades dealing with these pollutants. Rather than learn from this lesson, we have incorporated another halogen, fluorine, into numerous industrial, agricultural, medical, and consumer chemicals. This includes numerous pesticides and pharmaceutical compounds, which are continuously released into the environment and thus are present in sunlit surface waters. Upon direct or indirect photolysis in aquatic systems, persistent fluorinated byproducts or fluoride from pesticides and pharmaceuticals are formed depending on the type and stability of the fluorine-containing functional group. Byproduct quantification is required to fully evaluate potential impacts of these compounds, and our methodology uses a combination of quantitative fluorine nuclear magnetic resonance spectroscopy (^{19}F -NMR) and high-resolution mass spectrometry to track the persistence and transformation of fluorinated molecules. The ^{19}F -NMR measurements allow a mass balance on fluorine to be performed, and density functional theory calculations can be used to estimate NMR shifts of compounds found via mass spectrometry to allow their quantification. Some motifs are persistent, generating compounds that are polyfluorinated alkyl substances (PFAS), while others degrade to fluoride. More oxidizing conditions (e.g., reaction with hydroxyl radical) often leads to a higher yield of fluoride as an end product, but also trifluoroacetic acid which is persistent. These results will assist in the evaluation of the fate of the large suite of fluorinated pesticides and pharmaceuticals in the environment and in the development of pharmaceutical/pesticide structures to limit persistent byproduct formation.

3.16.T-02 The Role of Photochemistry in the Environmental Fate of a New Antimicrobial Peptide

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Antibiotics are molecules used to treat bacterial infections that have contributed to the increase of human life-span over the last century. Widely used in human and veterinary medicine, these drugs have led to the development of antimicrobial resistance (AMR), a major global health threat. Antibiotics and their transformation products can reach the environment via various pathways, and have impact on human and environmental health by developing AMR in the environment or by exerting other deleterious effects. Therefore, the environmental fate of antimicrobials needs to be characterized. Their fate depends on various natural attenuation mechanisms, including photodegradation. In order to tackle AMR, new efficient antibiotics less susceptible to create resistance are needed. A promising antimicrobial peptide, Pep16, was synthesized. Its environmental fate and its transformation products need to be characterized to ensure that the possible wide use of this compound will not present risks to human and environmental health. Photodegradation and water stability of these compounds were assessed in the laboratory in batch scale. Hydrolytic degradation was studied in deionized water and in sterilized Seine River water in the dark at different pH. Direct and indirect photolysis were investigated using solar sunlight. Solutions were placed in quartz tubes on the roof of Sorbonne University (Paris, France). Pep16 and its transformation products were analyzed via HPLC-HRMS. In deionized water, Pep16 was not hydrolyzed, although it was degraded in complex environmental matrix. Since the degradation did not follow pseudo first-order kinetics, it is likely that metals or inorganic species contribute to the hydrolysis of Pep16. Pep16 was not phototransformed at pH = 4 and pH = 7; however a 39% degradation was observed at pH = 9, associated with a low quantum yield determined via PNAP/Pyr actinometry. Indirect photolysis of Pep16 appeared to be faster than direct photolysis. IO_2 seems to be the main contributor in this degradation, hence further experiment using sensitizers and probes will be conducted to study it. Phototransformation products will be used to propose degradation pathways and their potential toxicity will be predicted using existing models. This work contributes to the evaluation of the environmental fate of Pep16, which is essential for assessing the potential impact of this compound on human and environmental health should it be commercialized.

3.16.T-03 Photochemical Degradation of Diclofenac and Cimetidine in Natural Aquatic Environments: A Comparative Study of Laboratory and Field Experiments

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Photochemical transformations are critical for mitigating pharmaceutical contamination in natural aquatic systems, yet translating laboratory findings to real-world conditions remains challenging. While the photodegradation mechanisms of compounds like diclofenac and cimetidine are well understood under controlled laboratory conditions diclofenac undergoing direct photolysis and cimetidine degrading through singlet oxygen-mediated indirect photolysis environmental variability complicates predictions. To bridge the gap between laboratory-based measurements and real-world observations, we investigated the photodegradation of these pharmaceuticals in an outdoor experimental wetland pond through four two-day field experiments. We added 10 µg L⁻¹ of diclofenac and cimetidine to the pond and monitored their degradation hourly, alongside measurements of water quality (dissolved organic carbon, UV-visible spectroscopy, and pH) and photochemical reactivity (singlet oxygen, hydroxyl radicals, carbonate radicals, and hydrogen peroxide concentrations). The field experiments revealed discrepancies between laboratory-based predictions and observed degradation rates, with diclofenac and cimetidine displaying half-lives of 48.3 (lab) to 73.5 (field) hours and 62.7 (lab) to 95.8 (field) hours, respectively. These slower observed degradation rates in the field suggest that environmental factors such as light attenuation, seasonal changes, and reactive species substantially influence degradation in natural settings. Notably, hydroxyl radicals contributed more significantly to cimetidine degradation than previously recognized. Our findings contribute to the broader effort to manage pharmaceutical contaminants in aquatic ecosystems more effectively by improving the accuracy of photodegradation predictions. In this presentation, we will discuss critical knowledge gaps in moving from the laboratory into the environment and the importance of refining predictive models to incorporate environmental variability.

3.16.T-04 POLITE: Photomodification of Low-Sulfur-Fuel-Oils, Investigations of Toxic Effects

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The 2010 Deepwater Horizon oil spill changed our understanding of the significance of photomodification as a fate pathway following an oil spill. It revealed a considerable portion of non-volatile, non-soluble surface oil was rapidly oxidized by sunlight, resulting in the formation of complex mixtures of tens of thousands of water-soluble oxygenated compounds. The toxicity of these photo-products is largely unknown, and their propensity to form varies with oil type, however for new fuels like very low-sulfur fuel oils (VLSFO) it is not known whether they will behave in the same manner as traditional crude and fuel oils.

The POLITE project sought to improve the understanding of the photomodification potential of VLSFOs and their relative toxicity to species that are important to aboriginal, commercial and recreational fisheries. We determined the toxicity of 13 VLSFOs both with and without photomodification to a variety of early-life stages of marine organisms including, American lobster (*Homarus americanus*), Atlantic cod (*Gadus morhua*), and green-sea urchin (*Strongylocentrotus droebachiensis*).

A total of 31 bioassays were conducted with 17 products, using low energy water accommodated fractions (WAFs, n = 150) that were prepared and mixed either in the dark or under full spectrum UV light exposures for an 18-hr period using an Atlas Solar Constant lamp.

Following irradiation, the exposure metrics for many of the products tested significantly increased, suggesting that there were photoproducts being formed. The amount of photoproducts formed varied between oil samples, with some of the tested VLSFOs not showing any detectable changes following irradiation. In nearly all cases the observed toxicity in the UV treated WAF was equal or greater than the WAF prepared in the dark. Larval lobster immobilization was the most sensitive endpoint across the tested species.

Using waterborne PAC concentrations, toxic units were calculated, and predictions were compared against the observed responses. The toxic units had good agreement with observed effects; however they were underpredicting the toxicity of many of the VLSFOs products, both with and without UV irradiation. These results suggest that there are other components within VLSFOs which are contributing towards the toxicity.

3.16.T-05 Next Generation PhotoFate: A Technique for Simultaneous Determination of Indirect Photochemical Degradation Rates, Transformation Products, and Second-Order Rate Constants *William Fahy and Scott A. Mabury, Department of Chemistry, University of Toronto, Canada*

Indirect photochemistry is an important transformation pathway for environmental contaminants in surface waters. However, the rates of indirect photochemistry can vary significantly between contaminants and surface waters depending on the reactivity towards and concentration of photochemically produced reactive intermediates such as hydroxyl and carbonate radicals, singlet oxygen, and excited triplet state organic matter. Significant strides have been made in understanding the reactivity of these species, but accurately measuring rates of indirect photochemistry still requires careful experiments to determine second-order rate constants with respect to each reactive intermediate, comprehensive assessment of the role of surface water components on indirect photochemistry, or sampling and testing of many genuine surface water samples. This variability and a lack of a standardized procedure for measuring the rates of indirect photochemistry has limited its inclusion in chemical fate and exposure assessments by regulatory bodies and industry.

Here we propose and validate a standardized method for assessing the role of indirect photochemistry in the fate of environmental surface water contaminants. Our approach couples a detailed kinetic model with a streamlined version of the PhotoFate technique, where the indirect photodegradation rates of a compound are tested in a series of synthetic surface water mixtures created by combining surface water constituents at various concentrations. The experimental half-lives in the synthetic surface waters provide a range of possible indirect photochemical reaction rates in surface waters globally. With the same dataset and the modelled steady-state concentration of photochemically produced reactive intermediates, second-order rate constants between the environmental contaminant of interest and each reactive intermediate can also be estimated by fitting to the experimentally measured pseudo first-order rate constants in each solution. Finally, a comprehensive and reproducible distribution of possible photoproducts can be identified from the synthetic surface water solutions. This technique is simple to perform and standardized to ensure results will be reproducible between laboratories, allowing interlaboratory comparison, benchmarking of the rates of indirect photochemistry in sampled surface waters, standardized testing of new compounds, and regulatory assessment.

3.16.P Photochemical Transformation of Contaminants in Aquatic Environments

3.16.P-Mo246 Sunlight-Responsive Photocatalytic Membrane for the Treatment of Emerging Contaminants

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The availability of clean water is a global concern due to the usage of multiple chemicals, which tend to be persistent in day-to-day life. Similarly, conventional water or wastewater treatment plants could not remove the recalcitrant and emerging contaminants completely and thus require advanced treatment. Photocatalysis is seen as one of the most advanced treatment techniques due to the stable and powerful oxidant generation that degrades pollutants. However, at the end of the treatment, it is tedious to collect the photocatalyst particles for reuse, which makes the treatment costly and unsustainable. These facts suggest attaching the photocatalyst particles to a surface and applying it for that purpose. In the same aspect, if the photocatalyst particles are merged with the membrane, the resultant photocatalytic membrane reactor (PMR) was observed to have the advantages of (i) complete utilization of photocatalysis; (ii) membrane-based filtration and photocatalysis as a single unit; (iii) no escape of particles, so no worry of having the nano-photocatalysts in the treated water. Having this understanding of the PMR, in this talk, the different configurations of the PMRs, operational parameters, and influencing factors on the treatment of emerging pollutants-contaminated water from different sources will be discussed.

The 2,4-dichlorophenoxyacetic acid (2,4-D) is an agrochemical used to remove weeds in paddy fields. Due to the toxicity associated, 2,4-D containing water needs treatment. Thus, herein, a sunlight-responsive ZrO₂-Bi₂O₃ photocatalyst was prepared by green synthesis using cow urine and tested for its efficiency in degrading 2,4-D. The prepared ZrO₂-Bi₂O₃ was then characterized in detail. The experimental results showed that 40% ZrO₂-Bi₂O₃ showed the best photocatalytic activity and stable even after five cycles of reuse. Furthermore, the sustainability of the studied technique that was analysed by the newly developed weightage-based ranking method showed that the present work addressed 14 of 17 SDGs. In addition, the mechanisms of different photocatalysts during the adsorption and catalysis stages that were observed in

the laboratory and how the performance and mechanisms vary with sunlight-based experiments compared to the controlled environment will also be explained.

3.16.P-Mo248 Understanding the Role of Photolysis in the Aquatic Fate of Antimicrobial Transformation Products

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The global proliferation of antimicrobial resistance (AMR) presents a complex concern, impacting the well-being of humans, animals, and the environment, within the One Health framework. Current environmental risk assessments of AMR primarily focus on antimicrobial parent compounds, with limited consideration given to their transformation products (TPs). However, antimicrobial TPs can be ubiquitously found in aquatic environments due to incomplete or even absent removal in effluent wastewater with conventional wastewater treatment. Thus, natural processes, such as photolysis, may substantially influence the distribution and persistence of these compounds in surface waters.

This study investigates the role of photochemical processes in the degradation of seven antimicrobial TPs derived from various antibiotic classes: hydroxy-metronidazole, hydroxy-trimethoprim, N-acetylsulfamethoxazole, clindamycin sulfoxide, erythromycin A enol ether, anhydro-erythromycin, and 4-epianhydrotetracycline. Experiments were conducted under controlled irradiation intensities (250 and 600 W m⁻², across 300-800 nm) to simulate different seasonal and/or altitudinal conditions, and considered different water matrices (MQ, surface and sea water) as well as the influence of other water parameters (e.g., pH, salinity, dissolved solids). All compounds were degraded to different extents over the course of the 56 h experiment. Most TPs followed first-order kinetics, with indirect photolysis and hydrolysis seemed to have a stronger influence than the irradiation intensity. Overall, several of the investigated TPs (N-acetylsulfamethoxazole, clindamycin sulfoxide, hydroxy-trimethoprim, and anhydro-erythromycin) have provided limited photodegradation potential, highlighting the persistence of these organic micropollutants in different water matrices, hence the importance of considering them in environmental risk assessments.

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3.16.P-Mo249 Photodegradation of Trifluoromethyl-Substituted Aromatic Compounds: Effect of Nitrogen Presence in Aromatic Rings

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Several pharmaceuticals and pesticides containing trifluoromethyl-aromatic moieties, such as fluoxetine, TFM, flupyr-sulfuron-methyl, and trifluridine, have faced regulatory scrutiny due to their classification within the large and problematic PFAS group. Some of these compounds photodegrade both directly and indirectly producing trifluoroacetic acid and fluoride anion as final products. To predict their degradation rates in the environment, assess the byproducts formed, and to ultimately design less persistent products, it is important to understand which structural features drive photochemical degradation. Here, we characterized the photodegradation of trifluoromethyl substituted aromatic and heteroaromatic model compounds, including benzoic acid, pyridine, pyrimidine, pyrazine, imidazole, pyrazole, and triazole. We determined their quantum yields and radical reactivity through a combination of direct and indirect photolysis experiments. Furthermore, using 19F NMR, mass spectroscopy and computational methods, we attempt to determine intermediates and final products formed by photodegradation and uncover the mechanisms at play. Our results reveal that an increased presence of nitrogen in the aromatic ring diminishes photodegradation rates. This reduction is due to a decrease in quantum yield, resulting in a lower efficiency of direct degradation processes. Moreover, an increased number of nitrogen atoms in the structure also hinders reaction rates with hydroxyl radicals. Additionally, we observe that the presence of nitrogen in the ring also increases the yield of trifluoroacetic acid and leads to less defluorination. The intermediate products formed and the final trifluoroacetic acid yield also depend on the reaction pathway, with indirect photolysis increasing trifluoroacetic acid formation for some compounds. Overall, these findings contribute to a deeper understanding of the photodegradation mechanisms of trifluoromethylated heteroaromatic compounds and highlight critical structural properties that may inform environmental risk assessment and regulatory considerations for such chemicals.

3.16.P-Mo250 Photochemical Degradation of Dimethylsilanediol in Fluvial Environments: Effects of Dissolved Organic Matter

Aleksandar I. Goranov¹, Jaeshin Kim², Debra Ann McNett², Marc-Andre Courtemanche² and Joseph Vasquez², (1)Old Dominion University, United States, (2)The Dow Chemical Company, United States

Dimethylsilanediol (DMSD) is the major degradation product of silicone polymers found in sealants, adhesives, lubricants, medical devices, cooking utensils, thermal insulation, electrical insulation, and other products. Interest in DMSD increased after it was detected in the potable water of the International Space Station. It is a challenging compound to quantify and thus, its concentrations and dynamics are poorly understood in the aquatic environment. This presentation will show findings from a comprehensive study of DMSD photochemical degradation in fluvial settings. Simulated sunlight was used to irradiate DMSD in water from four aquatic sources: Great Dismal Swamp (Virginia, USA), Suwannee River (Georgia, USA), Lake Ocklawaha (Florida, USA), and the Everglades (Florida, USA). Quantitative ¹H NMR spectroscopy determined that DMSD degrades following pseudo-first-order kinetics (i.e., rate = k [DMSD]), with the rate constants k being similar for the experiments with Suwannee River, Lake Ocklawaha, and Everglades media. The degradation of DMSD was much faster in the Great Dismal Swamp media likely due to the high iron loadings stimulating photo-Fenton oxidation of DMSD. NMR relaxometry (measurements of spin-lattice relaxation times, T₁, of DMSD) revealed that DMSD does not bind to fluvial molecules through non-covalent pathways such as hydrogen bonding or Van der Waals forces. Ultrahigh resolution mass spectrometry (10-Testa FT-ICR-MS) indicates the formation of new Si-containing compounds during photo-irradiation. This implies the formation of covalent bonding between DMSD fragments to fluvial organic molecules, however, this mechanism for DMSD removal appears to be insignificant in fluvial environments (as determined by kinetic experiments). Additional experiments showed that hydroxyl radical and superoxide species oxidize DMSD in significant amounts, suggesting these oxidation pathways as predominant. By contrast, it was revealed that DMSD did not degrade upon exposure to sunlight in pure water or to singlet oxygen species. Lastly, solid-state ²⁹Si NMR revealed that photo-flocculation may also be responsible for sequestering DMSD to some extent in fluvial settings. Collectively, these laboratory experiments expanded the knowledge on how DMSD degrades environmentally and further point to reactive oxygen species as responsible for DMSD losses in the environment (i.e., secondary photochemical degradation).

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3.16.P-Mo251 Comparative Investigation of Microplastic Fiber Formation from Five Synthetic Textile Fabrics Under UV-A Exposure

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Microplastic fibers (MPFs) are the dominant form of microplastics ubiquitously present in the environment, with textile fabrics being the largest source of MPFs. Though several research works have been conducted previously to understand the transformation of plastic products under photo radiation, not much research have been undertaken to investigate the fate of textile fabrics which is the major source for MPFs pollution. In Asian countries like India, a huge number of clothes are discarded every year in the water bodies as part of religious rituals, leaving the water body contaminated. These clothes eventually enter the ocean while being constantly exposed to sunlight. Therefore, to mimic this scenario, five different fabrics, namely polyester, georgette, satin, nylon, and lycra, were chosen for the study. These fabrics were cut into 5x5 sq. mm sized square and subjected to sacrificial photo radiation studies in aquatic suspension for 1, 3, 5, 7, 14, 21, and 46 days of UV-A radiation in an annular photoreactor fitted with a 400 W 365nm UV lamp. The fresh and aged fabrics were then characterized using X-Ray Diffractometer (XRD), micro-Fourier Transform Infrared Spectroscopy (μ -FTIR), and Scanning Electron Microscopy (SEM). Besides, after each experiment, the suspension media was completely filtered onto a Whatman glass fiber filter (GF/C, 47 mm diameter, 1.2 μ m pore size) to quantify the number of released MPFs. The entire filter was then observed under an optical microscope in a zig-zag manner to capture and characterize the whole set of released MPFs. Notably, nylon fabric has become brittle, eventually breaking down into tiny, uncountable numbers of particles at 7d of exposure. Among the remaining fabrics, the number of released MPFs was highest in satin (>1000 MPFs), followed by lycra, PET, and georgette. The obtained estimation correlates with the surface characteristics analysis, with satin showing a greater number of long and wider cracks compared to other fabrics. μ -FTIR analysis has confirmed the occurrence of aging, with aged fabric possessing varying intensity of peaks and peak shifts. However, no changes can be seen in the crystallinity of the aged fabrics, as confirmed by XRD analysis. Overall, the

outcome of this study would enable policymakers to understand the need for the management of textile fabrics that are discarded into the water bodies.

3.16.P-Mo252 Role of Direct and Sensitized Photolysis in the Photomineralization of Dissolved Organic Matter and Model Chromophores to Carbon Dioxide

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This study addresses the fundamental processes that drive the photomineralization of dissolved organic matter (DOM) to carbon dioxide (CO₂), deconvoluting the role of direct and sensitized photolysis. Here, a suite of DOM isolates and model compounds were exposed to simulated sunlight in the presence of various physical and chemical quenchers to assess the magnitude, rate, and extent of direct and sensitized photomineralization to CO₂. Results suggest that CO₂ formation occurs in a biphasic kinetic system, with fast production occurring within the first 3 h, followed by slower production thereafter. Notably, phenol model chromophores were the highest CO₂ formers and, when conjugated with carboxylic functional groups, exhibited a high efficiency for CO₂ formation relative to absorbed light. Simple polycarboxylated aromatic compounds included in this study were shown to be resistant to photomineralization. Quencher results suggest that direct photolysis and excited triplet state sensitization may be largely responsible for CO₂ photoproduction in DOM, while singlet oxygen and hydroxyl radical sensitization may play a limited role. After 3 h of irradiation, the CO₂ formation rate significantly decreased, and the role of sensitized reactions in CO₂ formation increased. Together, the results from this study advance the understanding of the fundamental reactions driving DOM photomineralization to CO₂, which is an important part of the global carbon cycle.

3.16.P-Mo253 The Role of Lipids in the Photoinactivation of Enveloped Viruses

Eliane Ballmer¹, Juliana Laszakovits¹, Alexandria B. Bohem² and Kristopher McNeill¹, (1)ETH Zurich, Switzerland, (2)Stanford University, United States

Aquatic ecosystems can serve as important reservoirs for enveloped viruses such as influenza viruses. Due to the possibility of virus transmission via contaminated waters, knowledge on the persistence of enveloped viruses in these matrices is decisive to better understand transmission pathways and risks. So far, it has been established that factors such as temperature, biological activity, virus subtype and water characteristics (e.g., pH, salinity) impact the decay rates of enveloped viruses. While only few studies have investigated the fate of enveloped viruses upon exposure to sunlight, recent experimental evidence suggests that enveloped viruses have lower photoinactivation rates compared to non-enveloped viruses, questioning the role of the envelope in the photoinactivation pathway. The envelope, consisting of a lipid membrane and associated glycoproteins, contains unsaturated lipids, which may be reactive towards photochemically produced reactive intermediates such as singlet oxygen. We therefore hypothesize that the lipid membrane presents a target for the photoinactivation of enveloped viruses in DOM-influenced surface waters. To test this hypothesis, we synthesize lipid vesicles of different lipid compositions and degrees of unsaturation via extrusion, expose them to sunlight in presence of DOM and trace their degradation using high performance liquid chromatography-mass spectrometry (HPLC-MS). By evaluating the photochemical degradation kinetics of the lipid membrane of enveloped viruses, our work aims at enhancing the understanding of virus persistence in aquatic environments, eventually allowing for better estimates of transmission routes and risks.

3.17 Combined Effects of Contaminants and Global Change Stressors in Estuarine and Marine Environments

3.17.T-01 Inferring Exposure to PFAS Over Time in the French Atlantic Coast by Sediment Core Analysis

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Per- and polyfluoroalkyl substances (PFAS) have been reported as widely distributed in oceanic environments. However, the occurrence of these highly persistent chemicals in the sedimentary compartment of marine regions is very little studied, despite the fundamental role of marine sediments in biogeochemical cycles, being both a compartment of storage of pollutants and the main route of exposure of benthic organisms. The study of vertical profiles of contaminants in sediment cores represents a unique retrospective environmental approach to infer their deposition and exposure history. In this work we (1) investigated the occurrence of PFAS in sediment core horizons covering the last 50-100 years in the Gulf

of Biscay (North-East Atlantic Ocean), and (2) assessed the PFAS historical and present concentrations and exposure trends in the study area by investigating their vertical profiles in the sediment cores. Three sediment cores were collected in sites subject to different anthropogenic pressures by using an interface corer equipped with stainless steel tubes on board of the R/V Thalia (IFREMER) in May 2022 (ROCCHSED2022, DOI: 10.17600/18002069.). Once in the lab, the cores were sliced into 1 cm horizons and dated. PFAS were microwave-extracted from the sediments, the extract cleaned-up by sequential solid phase extraction and PFAS quantified by isotopic dilution LC-ESI-MS/MS. A significantly higher loading of sumPFAS (Wilcoxon, $p = 0.0001$) was recorded in the accumulation area of sedimentary material drained by the Gironde River (west Gironde mud patch), where core 22-20 was collected, with median concentrations of $314 \text{ pg g}^{-1} \text{ dw}$. The fact that core 22-24 was sampled closer to the coast, in a touristic area and under the influence of some urban/industrial areas from Spain, did not seem to have a big influence in the median sumPFAS levels in the area, which were more than 6-fold lower ($\sim 50 \text{ pg g}^{-1} \text{ dw}$). The vertical profiles in core 22-20 allowed us to trace back the PFAS presence in the study area to the early 50 s. The first detections were observed in year 1952, although only one compound was found in this period and at very low concentration (few $\text{pg g}^{-1} \text{ dw}$). Even if the total PFAS concentrations increased by a factor of 30 since first detections to modern times, the PFAS fingerprint, and therefore the exposure pattern, seems to have been quite homogeneous over time. Perfluorooctane sulfonic acid (PFOS) was one of the predominant compounds across time.

3.17.T-02 Field and Laboratory Studies Assessing the Transport Behaviour of Priority Pollutants in the Tidal Elbe

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Sediment management in the Elbe estuary serves to expand and maintain the waterway. As fluvial Elbe sediments are contaminated, their relocation and natural transport can lead to pollutant accumulation as well as their widespread distribution. Old legacy pollutants can be distributed between water, suspended particulate matter (SPM) and sediment. The physicochemical dynamics that control the transport of pollutants between these compartments are sometimes inadequately reflected in prediction models. In order to generate more reliable data and optimize forecasts for the Elbe estuary, an interdisciplinary approach based on field and laboratory studies was used to investigate the distribution behavior of selected pollutants. These data are used to parameterize a new numerical model that integrates interactions between sediment, SPM and pollutant transport.

This contribution provides current and discrete data for legacy pollutants in the tidal Elbe SPM with a spatial distribution and corresponding KD values. The samples were collected on a sampling campaign with the research vessel Ludwig Prandtl in April 2023, prepared in the laboratory, digested according to Zimmermann et al. (2022) and measured via ICP MS/MS. In addition, monthly pooled samples provided by the Lower Saxony State Agency for Water Management, Coastal Defence and Nature Conservation (NLWKN) were subjected to sequential extraction according to the Community Bureau of Reference (BCR) to provide the model with valuable data on remobilization behavior under different environmental conditions.

The trace element mass fractions of the sediment samples range from $0.14 \pm 0.07 \text{ mg kg}^{-1}$ to $3.9 \pm 0.3 \text{ mg kg}^{-1}$ to $35.9 \pm 2.3 \text{ mg kg}^{-1}$ to $162 \pm 8 \text{ mg kg}^{-1}$ for Cd and Zn, respectively. The mass fractions of the SPM samples range from $0.26 \pm 0.16 \text{ mg kg}^{-1}$ to $3 \pm 0.4 \text{ mg kg}^{-1}$ to $83.0 \pm 10.0 \text{ mg kg}^{-1}$ to $552 \pm 29 \text{ mg kg}^{-1}$ for Cd and Zn, respectively. Overall measured mass fractions in SPM are significantly higher than the corresponding sediment samples. To support a precise modelling of pollutants in the Elbe catchment the data was used further to calculate KD values which are used as input parameters in various numerical models.

3.17.T-03 Distribution and Occurrence of Emerging Organic Compounds in the Estuary of Plentzia

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This study, conducted within the One Health framework, analysed contaminants of emerging concern (CECs) in the Plentzia estuary and Butroe River basin (Biscay, Basque Country) to understand their

distribution, occurrence, and role in coastal ecosystem health. CECs include diverse substances such as pharmaceuticals, PFAS, plasticizers, pesticides, and personal care products, many of which occur at trace levels but pose risks due to chronic exposure. Their varied physicochemical properties challenge their measurement and understanding of their environmental fate, requiring advanced analytical techniques like high-resolution mass spectrometry and extensive spatial and temporal monitoring. The research involved a year-long sampling campaign in the estuary and river basin, areas influenced by industrial activity, wastewater discharges, and significant seasonal population changes. Effluents from two wastewater treatment plants (WWTPs) were sampled alongside surface and bottom waters from various estuarine points, accounting for salinity and tidal conditions. Samples were filtered, extracted using Oasis HLB cartridges, and analysed on a Dionex UltiMate 3000 UHPLC coupled to a Q Exactive Focus quadrupole-Orbitrap (Thermo Scientific) equipped with a heated ESI source (Thermo, CA, USA). A selection of around 300 compounds, including many different CECs families, was used for a target analysis. A clear correlation was observed between WWTP effluents and estuarine waters, with similar contaminant patterns regardless of salinity or depth. The highest concentrations and frequencies of occurrence were found in intermediate salinity zones, where seawater dilution is reduced. Additionally, seasonal trends have also been observed, suggesting that the distribution may be influenced by temporal variations throughout the year. These findings highlight the need for continuous monitoring and advanced analytical approaches to address the environmental and health impacts of CECs in dynamic coastal systems, particularly under the pressures of human activity.

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3.17.T-04 Following the Mixtures of Organic Micropollutants with In-Vitro Bioassays in a Large Lowland River from Source to Sea

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Human-impacted rivers often contain a complex mixture of organic micropollutants, including pesticides, pharmaceuticals and industrial compounds, along with their transformation products. Combining chemical target analysis for exposure with in vitro bioassays for effect assessment offers a holistic view of water quality. This study targeted the River Elbe in Central Europe, known for its anthropogenic pollution exposure, to obtain an inventory of micropollutant contamination during base flow and to identify hotspots of contamination. We identified tributaries as sources of chemicals activating the aryl hydrocarbon receptor quantified with the AhR-CALUX assay, including historically contaminated tributaries and a newly identified Czech tributary. Increased neurotoxicity, detected by differentiated SH-SY5Y neurons cytotoxicity and shortened neurite length, was noted in some Czech tributaries. While effect-based trigger values (EBT) for oxidative stress response, xenobiotic metabolism and neurotoxicity were not exceeded, estrogenicity levels surpassed the EBT in 14% of surface water samples, posing a threat to fish reproduction. Target analysis of 713 chemicals resulted in the quantification of 487 micropollutants, of which 133 were active in at least one bioassay. Despite this large number of quantified bioactive chemicals, the mixture effects predicted by the concentrations of the quantified bioactive chemicals and their relative potency explained only 0.002-1.2% of the effects observed in the surface water extracts, highlighting a significant unknown fraction in the chemical mixtures. This case study established a baseline for understanding pollution dynamics and spatial variations in the Elbe River, offering a

comprehensive view of potential chemical effects in the water and guiding further water quality monitoring in European rivers.

3.17.T-05 Assessment and Prioritization of Aquatic Contaminants of Concern on Florida's Coral Reef

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Florida's Coral Reef (FCR) is the third largest barrier reef in the world and faces significant stress from localized, land, water, and air-based sources of pollution. Several chemical contaminants have been found in waters surrounding reefs and within corals, including pesticides, hydrocarbons, personal care products, pharmaceuticals, and trace metals. However, for most current and emergent environmental contaminants, little to no consistent information exists regarding environmental concentrations or toxicological effects on corals. Assessment of the levels of contaminants in coral reef environments is needed to conduct risk assessments to guide management, restoration, and recovery strategies, and the coral reef resource management community has recognized the need for improved, readily available comprehensive information.

Concentration data for metals, non-metals, and various organic compounds, consisting of over 72,000 marine data points for water and sediments, and covering over 800 sites in the five counties encompassing FCR (Broward, Miami-Dade, Palm Beach, Martin, and Monroe), were compiled from two main portals, the National Water Quality Monitoring Council Water Quality Portal and NOAA's National Status and Trends, and peer-reviewed literature. Concurrently, toxicological data for scleractinian corals was compiled from the EPA ECOTOX knowledgebase and peer reviewed literature for the same contaminant classes. These two datasets were then compared to establish existing knowledge gaps in both environmental monitoring data and toxicological effects on corals, and to identify any emerging contaminants of concern along the FCR. Where environmental data were not available, then potential sources and likelihood of occurrence for key contaminants were evaluated to estimate exposure risk, for example examining the most commonly used pesticides in Florida. The data, analysis, and evidence-based recommendations will support informed conservation management decisions and will be widely disseminated via incorporation into Florida's Department of Environmental Protection's (FDEP) coral reef decision support system (CRDSS).

3.17.P Combined Effects of Contaminants and Global Change Stressors in Estuarine and Marine Environments

3.17.P-Th252 Evaluation of Elevated Temperature Effects on Pollutant Toxicity in *Artemia salina*
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In the context of climate change, the increasing frequency and intensity of environmental stressors like marine heatwaves necessitate understanding their biological impacts, particularly when combined with pollutant exposure. This study investigates the acute toxicity of a landfill leachate sample collected from a leachate treatment plant in Crete, Greece, alongside two environmental pollutants, copper (Cu²⁺) and bisphenol A (BPA), on *Artemia salina nauplii* under ambient (25°C) and elevated (30°C) temperatures, simulating marine heatwave conditions. Mortality, expressed as lethal concentration 50 (LC50), and sublethal biochemical responses, including glutathione S-transferase (GST) and electron transport system (ETS) activity, were assessed.

At 30°C, LC50 values for Cu and BPA were significantly reduced compared to 25°C, indicating increased toxicity at elevated temperatures. For Cu, LC50 decreased from 104.94 mg/L to 79.27 mg/L, while for BPA, LC50 dropped from 54.73 mg/L to 10.71 mg/L. In contrast, leachate LC50 values were unaffected by temperature changes. Biochemical assays revealed that GST activity peaked at lower Cu concentrations and increased significantly with elevated temperatures, with activity doubling for Cu exposure at 30°C compared to 25°C. BPA exposure induced GST activity at the highest concentration tested, while no GST

response was observed for leachate exposure. ETS activity in control groups was temperature-sensitive, increasing significantly at 30°C; however, pollutant exposure did not significantly alter ETS activity at either temperature.

These findings show the complex interplay between temperature and pollutant toxicity, with elevated temperatures intensifying the adverse effects of Cu and BPA on marine organisms. The results highlight the vulnerability of marine and aquatic ecosystems to compounded stressors, suggesting that rising global temperatures and pollution may synergistically threaten biodiversity and ecosystem stability.

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3.17.P-Th253 Toxicological Evaluation of Plastic and Antifouling Paint Leachates on Two Life Stages of an Estuarine Copepod: *Nitokra spinipes*, in the Context of Global Change

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Plastic production soared to 400 million tons with a substantial proportion finding its way from inland areas to riverine and coastal environments. Within aquatic environments, plastics and biocide boat coatings undergo physical and chemical degradation, leaching chemicals from the polymer's matrix. These leachates have been found to have deleterious effects on aquatic organisms, such as copepods, crustaceans or fish. Furthermore, global change is currently increasing the frequency and magnitude of environmental stressors (i.e., increased temperatures), inducing negative effects in organisms. To the best of our knowledge, the combined effects of plastic-associated chemicals and global change remain largely unexplored. So, this study aims to evaluate the effects of leachates from plastic debris and boat coatings on *Nitokra spinipes*, a harpacticoid copepod key-species to estuarine food webs, in the context of global change. First, the most common observed plastics in an estuarine environment was established by sampling plastic items from the water column of the Iser estuary in Nieuwpoort, Belgium, via a manta net coupled to an aquatic drone; and from the banks using three quadrats of 50 x 50 cm randomly deployed along the tide line. After identifying polyethylene (PE) and polypropylene (PP) as the most abundant polymers as well as coating particles, leachates were produced using pristine PP and a Hemper's boat coating (7 days, at room temperature, in the dark). The toxicity of the leachates was then assessed by exposing *N. spinipes* adults and nauplii at 22°C and 25 °C (+3 °C, RCP 8.5 scenario, IPCC 2021). A significant toxicity was observed from the coating's leachates on nauplii development and mortality, whereas none was observed for the PP-leachates. Our results on adults showed a synergistic negative effect of the coating's leachates when combined to higher temperature (after 72 h, EC50 at 22°C = 44.7 ± 16.88% and EC50 at 25°C = 6.52 ± 10.73%). To conclude, coating's leachates exhibited significant toxicity in both life stages affecting greatly the adults when combined with elevated temperature. Our findings suggest that the predicted increase in temperature will aggravate the leachates toxicity on copepods and could lead to a cascading effect on the food web. Our results contribute to the risk assessment of plastic and coatings related litter in regional estuaries and help filling the knowledge gap on combined stressors effects on organisms.

3.17.P-Th254 Combined Effects of Plastic Pollution and Global Change on a Benthic Primary Consumer, *Nitokra spinipes*

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In estuarine waters, organisms are subject to multiple stressors in the environment, including anthropogenic pollutants such as plastic, and fluctuating physical-chemical parameters due to tidal and freshwater inputs. However, due to global change, physical-chemical parameters are predicted to be shifted towards new extremes in estuaries. For example, recurring extreme salt intrusion events will increase due to a higher frequency in heatwaves and draughts, more acidic water events will happen. Alongside these stressors, estuaries are considered reservoirs of plastic pollution, but the assessment of the combined effects of these parameters on primary benthic consumers, along generations, is lacking. Therefore, we aim to assess the combined effects of multiple stressors (pH, salinity, plastic particle forms) on a benthic estuarine primary consumer, *Nitokra spinipes*, across multiple generations. Using both

experimental results and coastal ecosystem knowledge, we aim to model the coastal ecosystem responses, already impacted by multiple stressors (e.g. fishery activities, pollutants, global change), to a shift in a benthic primary consumer group. We expect that sublethal effects will be observed when one of the physical stressors approaches the species tolerance limit, and that particle ingestion will potentiate these effects along generations. Expected effects can be the reduction of number of offspring to decrease or impaired growth and development, due to energy reallocation (food dilution) and population decline over multiple generations. Within the ecosystem, if the primary consumers are impacted, a cascade effect could be predicted with a shift in the access to food items on high food web levels, potentially leading to negative impacts on economic activities leaning on fisheries.

3.17.P-Th255 Small Microplastics and Microlitter Components (<100 µm) in the Invasive Blue Crab *Callinectes sapidus* from a Mediterranean Lagoon

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Small microplastics (SMPs <100 µm) can be ingested by organisms at the lower layers of the trophic web, leading to bioaccumulation and biomagnification in the higher trophic levels, with potential health risks associated with seafood consumption and the seafood industry. Additionally, the presence of MLCs (micro-litter components such as plastic additives, artificial fibers, and other micro-components), can further increase the potential threat to organisms, including humans. Invasive species, like the fast-growing blue crabs (*Callinectes sapidus*), originally from the western Atlantic coasts and now found in coastal environments like lagoons, may be particularly prone to ingest SMPs and MLCs, due to their predatory feeding habits and benthic lifestyle in transitional environments. Blue crabs (*Callinectes sapidus*) were collected from various sites in the Venice Lagoon in August 2024 as part of the PNRR spoke 1 Biodiversity project. The samples were dissected in a Clean Room (ISO7) and subjected to pseudodigestion process involving optimized mild pre-treatment, purification, and filtration procedures based on a previously established method. A rigorous Quality Assurance and Quality Control (QA/QC) protocol was followed throughout. Filters were analyzed using Micro-FTIR, enabling both quantification and identification of SMPs and MLCs. The specific methodologies employed for these samples proved crucial for targeting these emerging pollutants and providing reliable, replicable data. These preliminary findings will contribute to the limited knowledge of SMPs and MLCs ingestions by invasive species and shed light on potential health risks associated with their consumption, with implication for seafood and production.

3.17.P-Th256 Microplastic and Phthalate-Screening in Wild *Mytilus galloprovincialis*

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Additives associated with microplastics pose a double threat to the environment and aquatic organisms' health. Many of these compounds, such as phthalates, are classified among the most hazardous contaminants. These additives can be readily released into the environment, due to their lack of covalent bonds with synthetic polymers, potentially causing toxic effects, including immunotoxicity, neurotoxicity, and oxidative stress in exposed organisms. *Mytilus galloprovincialis* emerges from research as a potential bioindicator of microplastic pollution, playing a key role in monitoring the health of the marine ecosystem and the implications for human health. In this context, this study aims to detect microplastics and phthalates in the soft tissues of wild *Mytilus galloprovincialis*, evaluating a potential correlation between contaminants and the use of bivalve species to monitor microplastic and additive pollution. Several analytical methods were applied. On the one hand, the pre-treatment method was based on pseudo-digestion at temperatures below 40°C thus avoiding polymer degradation, which could lead to an underestimation of particle abundance. This methodological approach enabled the characterisation of microplastics with a high yield by micro-Fourier Transform Infrared Spectroscopy. On the other hand, the screening of phthalates was performed by Liquid Chromatography/High-Resolution Mass Spectrometry. Polyamide was the most widely detected polymer with a prevalence of ellipsoidal shape and an average length of 50.95 µm and width of 22.01 µm. The phthalate analysis detected Diisobutyl phthalate (DIBP) in all samples. The applied methodology allowed the identification and quantification of microplastics and phthalates in mussel tissues, providing indication on their possible sources and environmental fate, and

predicting the potential risks of transporting these contaminants throughout the food chain. Furthermore, *M. galloprovincialis* has great potential for monitoring microplastic pollution and exposure levels of organisms, allowing for the prediction of adverse effects on biodiversity and ecological stability associated with the presence of microplastics and additives in the marine environment.

3.17.P-Th257 Plastic Leachates Stimulate Feeding Responses in Sea Anemones

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Plastic pollution is accumulating in the marine environment and being consumed by marine animals. Marine animals are hypothesized to consume plastic in part due to the chemosensory cues emitted by plastic. We leached plastic (pre-production pellets and knives) in high-performance liquid chromatography (HPLC) water for 24 hours. Sea anemones (*Exaiptasia pallida*) were fed pre-combusted glass fiber filters with 7.5 µL of leachate and HPLC grade water (controls), and the time from ingestion to egestion was measured. Anemones fed filters with plastic leachates had feeding retention times 3.5 to over 4-fold longer than controls. Subsequent feeding of the same filters to new anemones resulted in decreased feeding retention times. We characterized the polymer of the knives and pre-production pellets used in the leachate with Fourier transform infrared spectroscopy and determined both were polystyrene. We used liquid chromatography-mass spectrometry to determine leachate chemistry but the concentrations of chemical compounds fell below detection levels despite concentrating the leachate 25-fold. This study provides evidence that molecules leaching from polystyrene pre-production pellets and knives contain feeding stimulants for sea anemones. Reducing the amount of plastic entering the ocean and limiting or altering flavors that leach from plastics may reduce plastic consumption by marine animals.

3.17.P-Th258 Polycyclic Aromatic Hydrocarbons Levels in Corals Exposed to an Oil Spill Simulation: Physiological and Ecological Implications

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To investigate the effects of oil pollution on coral health, a 72-hour exposure experiment was conducted utilizing the stony coral *Euphyllia paraancora*. The corals were exposed to varying concentrations of motor oil: 10 g/L and 1 g/L. The study focused on assessing the bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) in the corals and its impact on lipid content, density and chlorophyll levels of symbiotic algae (zooxanthellae). The results revealed a significant accumulation of PAHs in corals exposed to the higher oil concentration (10 g/L). Although coral lipid content remained largely unaffected by oil exposure, both the density and chlorophyll content of symbiotic algae showed significant declines under higher pollution conditions compared to the lower pollution group. These findings suggest that oil pollution may disrupt the symbiotic relationship between corals and the zooxanthellae, which could threaten coral survival and reef health.

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3.17.P-Th259 Examining Export and Bioaccumulation of Methyl Mercury in a Wetland Impacted by Avian Guano and Water Table Restoration

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Big Meadow Bog (Brier Island, NS) underwent water table restoration in 2018 that returned the bog to its original water levels prior to ditching. The Northern bog hosts a cluster of 4000-6000 herring gulls that

feed at nearby mink farming and aquaculture sites. Previous research in our group has shown that bird biomass and waste products are increasing the trace metals and nutrients measured in groundwater. Analyses have shown that gull guano is very high in water soluble PO₄³⁻ but low in total mercury (THg) and methylmercury (MeHg). Research from various studies examining terrestrial freshwater catchment flooding indicates that MeHg increases over a multi-year time span. An examination of water quality and lower trophic level biota was initiated to set a baseline for MeHg concentrations in the base of the food web. Water from the primary surface outflow was collected over five years (2018-2023) and invertebrate samples were collected over three summers (2021, 2022, and 2023). Mean concentrations of MeHg, DOC, PO₄³⁻, and NO₃⁻ in water were significantly higher in the first-year post-flooding and decreased through subsequent years. MeHg, DOC, and NO₃⁻ concentrations were all strongly correlated and significantly higher in the summer months than the winter months. MeHg concentrations in water were correlated with those in resident Gammaridae (benthic invertebrate) tissues, suggesting that this environment is conducive to MeHg bioaccumulation in gammarids. Recent installation of hydrological weirs to track surface outflow volumes show hypereutrophic concentrations of nutrients and elevated MeHg at the weir nearest the gull colony. Mercury dynamics in this local system are complicated by biovector introduction of various elements as well as past and recent anthropogenic activities, but serve as a case study for coastal wetland habitats situated near various types of industries.

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3.17.P-Th260 Methylmercury Concentrations of Freshwater and Coastal Invertebrates from the Jijuktu'kwejk Estuary, Nova Scotia, Canada

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Methylmercury (MeHg) is a toxic contaminant that bioaccumulates and biomagnifies in food webs; however, differences in mercury (Hg) retention and bioaccumulation between freshwater and coastal environments are not well understood. Inland rivers and estuaries transfer Hg and MeHg from anthropogenic terrestrial sources to coastal environments where MeHg accumulates in sediments and food webs. Identifying sediments and organisms with high Hg accumulation within an ecosystem is critical to the assessment of MeHg biomagnification in sensitive areas. This study examines the Jijuktu'kwejk estuary, Nova Scotia, Canada. Invertebrates, porewater, and sediment samples were collected from freshwater, estuarine, and coastal sites to assess environmental contamination. A total of 37 invertebrate families were collected. Apart from two families, estuarine invertebrates were of coastal origin. Mean MeHg concentrations in both coastal and estuarine invertebrates (13.2 ± 11.7 ng/g dry weight (dw) and 12.3 ± 11.4 ng/g dw, respectively) were ~10 times below the Canadian guideline for the protection of wildlife consumers (174.3 ng/g dw for 81% moisture). The mean MeHg concentration for freshwater invertebrates (125.2 ± 116.9 ng/g dw) was much higher, with two groups, water striders (Gerridae spp.) and a freshwater mussel, above the guideline (213.1 ± 223.0 ng/g dw and 191.8 ng/g dw, respectively). Several other freshwater invertebrate groups had mean MeHg concentrations near the guideline, including fishing spiders (*Dolomedes* spp.) (168.4 ng/g dw), water boatman (Corixidae) (153.9 ± 27.8 ng/g dw), and damselflies (Zygoptera) (150.5 ng/g dw). Mean MeHg concentrations for coastal and estuarine invertebrates did not exceed 40 ng/g dw, though species with the highest concentrations for these locations were bamboo worms (Maldanidae) (37.43 ng/g dw), eastern mudsnails (*Ilyanassa obsoleta*) (29.86 ± 16.45 ng/g dw), and European green crabs (*Carcinus maenas*) (28.09 ± 2.20 ng/g dw). These values in combination with stable isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ will allow us to compare MeHg bioaccumulation slopes for invertebrates between freshwater, estuarine, and coastal environments. Though further analyses for porewater and sediment Hg concentrations are needed, these initial results suggest that MeHg accumulation in low-trophic freshwater food webs is significant compared to that of estuarine and coastal environments within the Jijuktu 'kwejk estuary.

3.17.P-Th261 Progressing Short-Term Methods to Estimate Chronic Marine Toxicity for Regulatory Use in the North-East Atlantic Region

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Introduction

Within the North-East Atlantic region, reliable risk- and hazard-assessments are crucial for the management and permitting of offshore chemicals, produced water and other wastewater discharges. Assessments mostly rely on regulatory required acute Whole Effluent Toxicity (WET) and chemical toxicity tests, performed with a limited number of species, potentially leading to overly precautionary risk estimates due to the application of conservative assessment factors

Chronic toxicity data potentially reduce this uncertainty, but application of such data in regulatory context is limited, e.g. not regulatory required, practical limitations, availability and associated costs.

Risk-based approaches increase demand for chronic marine toxicity data to reliably assess chemicals and wastewater discharges both offshore and from land-based sites to coastal environments. There is a need to further develop and deploy short-term tests to assess chronic marine toxicity for regulatory use for both WET and chemical testing.

Objective

Identify suitable methods for estimating chronic marine toxicity based on existing protocols and global practices that would be applicable to the management of both offshore and coastal chemical use and discharges.

Deliverables

A set of well validated protocols that estimate chronic toxicity, selected based on their availability, regulatory acceptability and efficiency in terms of costs and executability.

Implementation Plan

- Review and analysis of existing protocols
- Protocol selection and testing plan design
- Pilot testing
- Dissemination

3.17.P-Th262 Occurrence of OPEs and PFAS in Surface Sediments from the French Atlantic Coast and Loire Estuary

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Organophosphate esters (OPEs) flame retardants and plasticizers and per- and polyfluoroalkyl substances (PFAS) are among the most widely distributed contaminants of emerging concern (CECs) in the marine environment. Both CECs reach marine coastal areas at high rates due to their non-stop emissions. There is high global concern due to their hazardous properties. While no regulations are in place or planned to limit production and emissions of most OPEs, a global restriction process has just been launched at European level by the European Chemicals Agency (ECHA). However, the occurrence of these CECs in marine sediments, considered global sinks of many organic contaminants, are still little studied compared to other environmental compartments. In this work we investigated the occurrence of parent OPEs (tri-OPEs), their major degradation metabolites (di-OPEs), and PFAS in various sampling locations along the French Atlantic coast (Gulf of Biscay), including the Loire estuary. The selected sites are under the influence of different anthropogenic pressures. Sediments were collected in intertidal areas from the Loire estuary in February 2022 and along the coast on board of the R/V Thalia (IFREMER) in May 2022 (campaign ROCCHSED2022, DOI: 10.17600/18002069.) One gram of dry material was extracted by sonication (OPEs) and microwave (PFAS). The extracts were cleaned up by solid phase extraction and quantified by isotopic dilution LC-ESI-MS/MS. PFAS were detected in all samples, with values varying from 0.03 to 0.6 ng g⁻¹ dw (?PFAS) in sites along the coast. Tri-OPEs were detected in 93% of samples and exhibited one order of magnitude higher concentrations than PFAS, with levels ranging from 0.2 to 2.8 ng g⁻¹ dw (?tri-OPEs) in coastal sites. Di-OPEs showed lower detection frequency (73%) and concentrations. Higher levels of both PFAS and OPEs were found in sites from the Loire estuary under harbour and/or industrial activities. Our results show the overall occurrence of both families of CECs pointing to industrial areas inside the estuary as relevant sources. Tris(chloro-2-propyl) phosphate (TCIPP), tris(2-ethylhexyl) phosphate (TEHP), and perfluorooctane sulfonic acid (PFOS) were among the most abundant CECs found in the study area. The influence of the sediment total organic carbon and granulometry on their spatial distribution in the study area is under evaluation.

3.17.P-Th263 Fate and Behaviour of Contaminants in Estuarine Systems: An Example from Southampton Water, UK

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Estuaries are important, often heavily urbanized ecosystems, acting as sinks for contaminants and moderating their input into marine environments. Many of these contaminants, including legacy contaminants and contaminants of emerging concern (CECs), pose significant ecological risks. The heterogeneous composition of estuarine sediments and the variety of contaminant groups make predicting their fate and behaviour in estuaries challenging, especially under changing environmental conditions. This study examines the role of estuarine sediments in contaminant adsorption processes by assessing CECs and conventional contaminants in an estuary and conducting controlled batch adsorption experiments.

Surface sediment samples and a sediment core were taken from Southampton Water, a large estuary in southern England. The spatial and temporal distribution of trace metals, polycyclic aromatic hydrocarbons (PAHs), and hormones were assessed using x-ray fluorescence analysis and targeted gas chromatography mass spectrometry (GC-MS). In addition, non-targeted analysis was conducted with a two-dimensional GC-MS. Sediment properties including total organic carbon and stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) were measured. Furthermore, the adsorption patterns of hormones on different types of estuarine organic matter at varying salinities are investigated.

Results of the estuarine samples show increasing contaminant concentrations close to point sources such as the port (e.g. Hg up to 2.4 mg/kg, PAHs up to 5600 ng/g). The dated sediment core documents historical waste discharges into the estuary over the past 60+ years. Core profiles from trace metals (e.g. Cu, Ba, Pb) show distinct discharge events, whereas organic contaminants (e.g. PAHs, Bisphenol A, phthalates) are present throughout the depth of the core, indicating their persistence and potential mobility. Hormones such as estrone were detected at concentrations between 1.8–13.2 ng/g in surface samples, but no trends or correlations with sediment properties were observed. Batch adsorption experiments are ongoing to give a more detailed insight into the adsorption behaviour of hormones onto different types of estuarine organic carbon under changing salinities.

This work will help to better understand hormone adsorption mechanisms under changing environmental conditions and contribute to a more comprehensive understanding of contaminant dynamics in estuarine systems which is crucial for effective risk assessment and management.

3.17.P-Th264 Prioritisation of CECs in the Marine Environment Using Hazard-Based Filtering and Ranking Approaches

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The increase in the use and production of chemicals has led to a growing number of contaminants of emerging concern (CECs) in the marine environment. A prioritisation strategy is necessary to study the environmental occurrence and effects of the most impactful chemicals in the marine ecosystem, guiding decisions on which compounds should be included in routine monitoring. To address the lack of marine-specific prioritisation schemes, a hazard-based prioritisation tool was developed for CECs within the European Union (EU) project An Integrated Approach for Assessing Impacts on the Marine Environment (CONTRAST). A comprehensive literature review identified the most frequently used criteria for prioritising CECs. Subsequently, the outcomes of an internal survey among project partners on the most important criteria to be included in the prioritisation scheme were used as a basis. CECs were filtered using three parallel schemes: persistence and bioaccumulation (PB), toxicity (T), and persistence and mobility (PM) using cut-off values adapted from the EU regulatory framework for PBT and PMT assessments. The filtering of chemicals was aided by a tool developed in the PikMe project, which was funded by the Norwegian Environment Agency. The PikMe tool contains a database regarding physical, chemical, and PBT properties, tonnage, and EU regulations of approximately 1.1 million chemical substances gathered from different data sources. The filtered chemicals were assessed further and scored based on their modes of action, occurrence in the marine and freshwater environments, and emission. A ranking based on the final score resulted in a list of around 8500 chemicals with high diversity regarding their functional use (e.g., pharmaceuticals, flame retardants, plasticisers, personal care products, and

biocides). The top 100 contains the compounds that will be selected for use within CONTRAST for the distribution, fate, and effects studies, determining their impacts on the marine biodiversity and providing an effective integrated monitoring framework to monitor their impacts on marine life.

3.17.P-Th265 A Paleolimnological Assessment of Environmental Degradation and Legacy Pollutants in a Back-Barrier Lagoon at Pictou Landing, Nova Scotia, Canada

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Sitmu'k is a marine lagoon located in an Indigenous community in Nova Scotia, Canada. Boat Harbour is a former estuary located 2 km from Sitmu'k that was converted to a pulp mill and chlor-alkali plant effluent impoundment in 1970. Boat Harbour contains > 577,000 m³ of contaminated sediment including metal(loid)s and a variety of persistent organic pollutants. A limnological assessment of Sitmu'k indicated significant water quality degradation, which residents suspected was associated with Boat Harbour effluent. A paleolimnological assessment was undertaken to provide a spatiotemporal perspective on legacy pollutants. Sediment gravity cores were collected from Sitmu'k and Boat Harbour and the estuary at the outlet of Boat Harbour, two nearby estuaries and a freshwater pond served as reference sites. Sediments were characterised using X-ray fluorescence (XRF); total Carbon (C), total Nitrogen (N) and stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes.

Boat Harbour effluent contains elevated Cu, Zn, Pb that increase up-core and exceed marine sediment guidelines, top-bottom core analyses indicated elevated geogenic As, also present at other sites in the study. Most Cu, Zn and Pb bioaccumulated in the pulp feedstock. Cores obtained at the outlet of Boat Harbour also contained elevated metals that decline seaward. All sites in this study contained elevated Pb, a legacy atmospheric pollutant from regional coal fired power generation and steel production. Total C, N and stable isotope values for the effluent reflect a terrestrial carbon source. In contrast, isotope analyses at Sitmu'k and reference estuaries indicated marine carbon sources and metal concentrations that decrease up-core and do not exceed guidelines. Elevated $\delta^{15}\text{N}$ in Sitmu'k sediments compared to all other sites is likely associated with residential development and effluent from local water treatment. Landward migration of the barrier-beach complex and reduction in water depth and circulation likely contribute to water quality degradation. Collectively the paleolimnological data indicate that the pulp mill and chlor-alkali plant effluent is not impacting Sitmu'k. This study demonstrates that the paleolimnological approach coupled with the use of reference sites can provide clarity on the impact of a variety of legacy pollutant sources in dynamic marine environments.

3.18 Chemical Emissions and Associated Environmental Impacts from Offshore Energy Production

3.18.T-01 Chemical Emissions from Offshore Wind Farms: Compound Identification and Prioritisation for Risk Assessment

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Offshore wind farms (OWFs) are a source of chemical emissions to the marine environment. A wide variety of compounds can enter the marine environment, from inorganics to dissolved organic chemicals and even plastic polymer particles. Before characterising the extent, distribution and impact of non-particulate emissions, it is essential to identify the individual compounds that can be emitted and to prioritise those compounds that might exert the largest negative impacts to the marine ecosystem and humans consuming marine products. Here, we provide a comprehensive overview of potential chemical emissions from OWFs, using a combination of literature data and non-target screening (NTS) by GCxGC-HRMS of paint leachates. This resulted in a long list of >400 individual chemicals representing a wide variety of compound groups including metals, alkylphenols, bisphenols, diisocyanates, alkyl and aromatic amines, diamines, benzotriazoles, organophosphate esters and BTEX compounds. Additional data was gathered, including information on the properties of concern at regulatory level (e.g. priority listing, classification) and the frequency of the release (intermittent vs continuous) to set a list of 51 candidate priority compounds. A second prioritisation step based on persistence, bioaccumulation, toxicity (PBT) and endocrine disrupting (ED) properties on humans and aquatic organisms was performed on the candidate list; leading to a categorization into (1) officially recognised PBT and/or ED substances, (2) potential PBT/ED substances or equal level of concern, (3) not PBT/ED but medium level of concern

substances and (4) not PBT/ED and low level of concern substances.

The combined literature overview and GCxGC-HRMS NTS offers valuable insights into the potential chemical emissions from OWFs. However, it is only a first step to identify the substances of potential concern. The prioritisation exercise based on the PBT and ED properties of the substances allows us to focus on substances that require a full risk assessment of OWF chemical emissions. Although the properties of these compounds are sometimes well-defined, we have already identified the need for additional data on bioaccumulation in aquaculture products and on ecotoxicity. Determination of environmental concentrations at OWFs will be necessary to complete the risk assessment of chemical emissions on the marine ecosystem.

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3.18.T-02 Untargeted Screening of Marine Sediments in Offshore Wind Farms: Tracking Chemical Emissions

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Offshore wind farms (OWFs) play a crucial role in reducing carbon emissions and the dependency on fossil fuels. The biological effects of OWFs on the marine ecosystem have systematically been monitored. However, chemical emissions have often been overlooked. As it is expected that current OWFs will further be expanded to comply with European energy and climate goals, it is paramount to understand and characterize the potential chemical emissions of these OWFs. This is not an easy task, as many activities are taking place in a limited area at sea, rendering it difficult to identify the specific emission sources. Sediment samples were taken in and near four OWFs within the Belgian and German parts of the North Sea to identify potential emissions. Three types of reference locations were considered: offshore reference samples in an area where low human impact is expected, aiming to capture background concentrations; ship reference samples, taken offshore near a shipping lane, aiming to correct for effects of ship traffic; and near-shore references, where a larger impact of multiple human activities taking place within 12 nautical miles of the shoreline can be expected. Sample extracts were analyzed on a Vanquish Orbitrap Exploris 120 LC-HRMS(/MS) and on a GC-triple quadrupole MS, allowing for the detection of a wide variety of compounds and covering both the polar and non-polar chemicals. More than 150 compounds were detected by GC-MS. Most of these compounds were present in both impact and reference samples. To identify compounds that might be leaching from OWFs, the relative intensity was calculated between impact and reference samples, and peaks with an intensity of at least five times the measured intensity at all three reference locations were withheld. Besides using the relative peak intensities, machine learning was also applied to identify potential peaks. A random forest model, for example, allows the identification of peaks that are associated with impact, nearby, or reference areas. After building a random forest model, the most important compounds that contributed to the impact or nearby areas were also selected. For the Belgian part of the North Sea, this consisted of more than 30 potential compounds (for the GC-MS method). Further identification with MS libraries allowed us to identify, to the greatest extent possible, these compounds and characterize, for the first time, the potential chemical emission of OWFs.

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3.18.T-03 Offshore Chemical Emissions (OffChEm): Six Years of Environmental Research In and Around Offshore Wind Farms in the German North Sea

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Offshore wind farms (OWFs) are pivotal for fulfilling the EU's goal to a sustainable energy transition and achieving climate neutrality by the year 2050. Different corrosion protection systems are commonly applied, including galvanic anodes, which dissolve over time and could therefore be a relevant source of metal emissions to the marine environment related with OWFs. Tracer elements for OWF-induced emissions from galvanic anodes consisting of six elements: Al and Zn as main components, the technology-critical elements (TCEs) In and Ga and as ecotoxicologically relevant legacy pollutants: Cd and Pb, have been previously published.

Sediment and water samples were taken over six years in the German North Sea. Sediments were processed by microwave assisted acid digestion following the protocol of Zimmermann et al. and subsequent multi-element analysis by an inductively coupled plasma tandem mass spectrometer (ICP-MS/MS) (Agilent 8800, Agilent Technologies, Tokyo, Japan).

Water samples were pressure filtrated and acidified with double sub-boiled nitric acid. An online preconcentration and matrix removal system was coupled online to an ICP-MS/MS system (Agilent 8900, Agilent Technologies, Tokyo, Japan) for quantification of the water samples.

The data show that mass fractions of the selected tracer elements in sediment are currently predominantly within the range of the known variability in the study areas. Locally elevated mass fractions were found occasionally in the sediment, especially for Pb and Cd. Based on the Ga and In elemental mass fraction data and the prevailing dilution and distribution processes, there are currently no direct effects discernible due to the use of galvanic anodes.

Elevated In concentrations were found in this study, while Ga concentrations were found to be within the known variability. The combination of In concentrations measurements, Gd anomaly calculations and drift trajectory modeling of water masses showed a high potential for an in-depth source assignment. The release of In caused by the dissolution of galvanic anodes seems to be associated to OWFs corrosion protection for at least one area within two different years.

Overall, the data do not prove direct impact of OWF emissions on elemental mass fractions, but galvanic anodes are per se a continuous source of metal emissions and thus are a new source of pollution within the marine environment.

3.18.T-04 Anticipate the Environmental Impact of Brine from the Desalination Process for Offshore Green Hydrogen Production on the Fish Model, *Scophthalmus maximus*

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In the pursuit of enhancing energy production from Offshore Renewable Energies (ORE), the production of hydrogen (H₂) integrated within the large-scale deployment of Offshore Wind Farms (OWF) appears to be a promising green energy vector. At sea, producing H₂ through freshwater electrolysis necessitates seawater desalination, which results in chronic brine discharge. This brine, consisting of hypersaline, warm water for the thermal desalination process, contains chemical residues, such as phosphonates and chlorination by-products. Discharging this brine into the marine environment induces local physicochemical changes, exposing marine organisms to osmotic, thermal, and chemical stress. In the context of the environmental integration of OREs, the environmental impact of chronic brine discharge into the offshore ecosystem is, to date, inadequately documented to anticipate potential environmental effects. The R&D project OPHARM2 aims to conduct an environmental impact assessment of the offshore H₂ production through a synergistic and transdisciplinary approach with three objectives: (1) evaluating acute toxicity across multiple trophic levels; (2) investigating the intrinsic and chronic toxicity of brine through cellular and sublethal approaches on a fish model; (3) mapping exposure gradient to brine discharge within OWFs using multi-scale hydrodynamic modeling.

This study presents preliminary acute toxicity results on the benthic model fish species, *Scophthalmus maximus*. These results allow us, on the one hand, to estimate the impact of salt stress through the LC10 of exposure to brine at different temperatures (18°C to 30°C) and, on the other hand, to identify the ecotoxicological specificities of each brine constituent (salt and chemicals) via a cellular approach on two key organs (gill and liver). This comprehensive and ecotoxicological approach offers the first data and insights

on the offshore toxicity of brine, enhancing the knowledge of the potential environmental impact of offshore green H₂ production.

3.18.P-Mo259 Ecological Impacts of Wind Turbine Blade Erosion on Blue Mussels (*Mytilus edulis*)

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Offshore wind farms (OWFs) introduce novel anthropogenic pressures to marine ecosystems, including the release of organic and inorganic particles from turbine blade erosion, which may impact marine life. This study investigated the potential effects of these particles and their additives on the metabolic profile of the blue mussel (*Mytilus edulis*) using 1H-NMR spectroscopy. Mussels were exposed in the laboratory to microplastic (MP) particles derived from cryo-milled rotor blade coatings and core materials (glass fiber polymer, GFP) under a simulated worst-case scenario of extensive blade erosion. Enrichment factors (EFs) for metals and metalloids (e.g., Ba, Cu, Cd, Cr, Ni) ranged from 0.93 to 6.1, indicating a potential chemical load from the rotor blade materials. Untargeted metabolic profiling of mussel tissues revealed no significant disruptions to metabolic pathways, however trends in metabolite levels suggested potential short-term effects on neuroendocrine systems and possible long-term impacts on energy metabolism. Further research is essential to comprehensively evaluate the potential impacts of OWF erosion on marine environments, particularly in light of the planned expansion of wind farm construction as part of the EU-wide energy transition.

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3.18.P-Mo260 Analysis of Coating Particles from Offshore Wind Farms in Sediment Samples

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Offshore energy production has risen sharply in the last decades. Consequently, the number of offshore wind farms (OWFs) is growing and chemicals may be released by the construction and operation of these structures. However, resulting impacts on the marine environment are still largely unknown. Within the EU Interreg project Anemoui, chemical emissions from OWFs and their effects are investigated. One source of emission is the particulate release of corrosion protection coatings and paints, as well as leading edge erosion of rotor blades leading to an introduction of microplastic particles into the marine environment.

Currently, only a small number of studies have investigated these paint and coating particles in marine samples. One reason for this is methodical challenges to analyse these particles in environmental samples and therefore, missing adapted protocols.

We tested different methodical approaches for separating paint and coating particles from OWFs from the sample matrix. On the one hand, different digestion protocols were compared. On the other hand, the density separation to separate the particles of interest from the sediment was adapted and improved. To do so, sediment (sandy or silty) was spiked with coating particles derived from six coatings commonly used on offshore wind turbines (four epoxy-based and two polyurethane-based) from two manufacturers. Two salt solutions with different densities were used and recovery rates calculated. Furthermore, procedures for the identification of particles by FTIR (ATR and FPA) and Pyrolysis GC/MS were optimized for the analysis of paint and coating particles and both approaches were compared. Finally, environmental samples from the North Sea were analysed using the developed protocol.

Tests showed that digestion chemicals have to be chosen with caution not to cause any harm to the paint and coating particles. With respect to the density separation, the solution with higher density resulted in higher recovery rates for paint and coating particles. The optimization of identification methods especially included the development of an adapted library for FTIR spectra of paint and coating particles. The adapted protocol was successfully applied to a first sample set of North Sea sediment samples.

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3.18.P-Mo261 Settling Velocities of Particle Emissions from Offshore Wind Farms

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The technology of offshore wind is undergoing a period of rapid growth, with an enormous expansion of installed capacity expected in the coming decades. To protect offshore wind turbines against corrosion, the structures are coated with organic coatings, which deteriorate over time and subsequently detach, either through corrosion or by accidental damage during operation. The environmental impact of these detaching coatings is not investigated yet. In order to address this knowledge gap, this work focuses on the investigation of the transport of coating particles through the water column by investigating the terminal settling velocity of particles manufactured from common coating materials. Particles from epoxy and polyurethane were made as single and double-layered particles with dimensions ranging from 1 to 3 cm in flat, regular shapes such as disks and squared plates. The particles were released into an acrylic glass tank in order to determine their terminal settling velocity, using both fresh and saltwater. The double-layered particles exhibited the highest settling velocity, followed by epoxy particles, and polyurethane particles, which demonstrated the lowest settling velocity. The experiments at different salinities showed that salt water as surrounding fluid led to a decrease in settling velocities. The findings of this study represent a significant initial step towards a more comprehensive understanding of the environmental transport of corrosion protection coatings and can inform the development of strategies to mitigate adverse environmental effects associated with all types of coated marine artificial structures.

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3.18.P Chemical Emissions and Associated Environmental Impacts from Offshore Energy Production

3.18.P-Mo254 Regulation of Chemical Emissions from Offshore Wind Farms: An Overview and Comparison for the North Sea Region

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Due to great environmental goals to increase the production of renewable energy and its advantages, offshore wind energy is a fast-growing sector. The current rise in construction of offshore wind farms (OWFs) in Europe makes comprehensive regulations for their construction, operation and decommissioning necessary to monitor and minimize potential effects. However, it is the task of national and/or local authorities to regulate OWFs which may result in differences between countries and missing overarching rules. In addition, some potential effects of OWFs are still largely unknown and are therefore difficult to be addressed in current regulations. For example, effects of chemical emissions from OWFs are not yet fully understood but research has already started. Effects on the marine environment of chemicals released from different parts and processes of OWFs cannot be excluded and should consequently be considered in regulatory documents to provide guidance for operators and limit possible negative environmental effects in advance.

National regulations for OWFs were reviewed and analyzed with respect to rules for chemical emissions from these structures (e.g. during approval/licensing procedures and the operational phase). The focus was on EU countries bordering the North Sea, including Norway. In a first step, national authorities and stakeholders were contacted to gather information on existing regulations in the respective country. In a second step, OWF regulations for chemical emissions were evaluated in detail and analyzed for the first positive steps and gaps in order to compare countries and identify similarities and differences.

The analysis showed that, overall, regulations for chemical emissions from OWFs are not yet fully developed even though first attempts have been made. Also, major differences were found between the countries with respect to the level of detail describing the conditions and limitations related to the use of chemical compounds and focus on the effects of OWFs to the marine environment. Our assessment reveals the need to increase the knowledge on applied techniques and effects of chemical emissions from OWFs to improve rules and accelerate the development of guidelines practicable for both environmental protection and OWF operators.

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3.18.P-Mo255 Techniques to Avoid or Minimize Chemical Emissions from Offshore Wind Facilities
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Offshore wind energy is a key element in the renewable energy sector and essential to reduce greenhouse gas emissions in Europe. The entity of Offshore Wind farms (OWFs) consists of different offshore facilities like offshore wind turbines for energy generation and offshore platforms such as substations and converters (HVDC stations) for energy collection, transformation, and subsequent energy transmission to shore by sea cables. All offshore facilities are complex technical structures, consisting of many various components and technical systems. To sustain their operability the use of operating materials and techniques is required which can lead to chemical emissions into the marine environment by accidents and in some cases also during regular operation. Impact on the marine environment can be minimised if offshore wind facilities are designed and conducted in a manner that they do not cause any avoidable emissions (precautionary principle). This approach can be achieved by using state of the art techniques. However, when it comes to emission control it is not easy to define state of the art techniques explicitly for offshore wind facilities, as only very few detailed recommendations or guidelines for OWFs are currently available but no best available techniques (BAT). However, techniques to avoid or minimize emissions from other (maritime) industrial fields (e.g. shipping, offshore oil and gas industry) may be appropriate and are already used to some extent.

Here, we present a comprehensive overview of relevant emission sources of chemicals into the marine environment by OWF and how chemical emissions can be avoided by using state of the art techniques (e.g. for cathodic corrosion protection, seawater cooling systems (biocides), sewage). This overview could be used as a first guideline for planners, windfarm operators, and administrations.

3.18.P-Mo256 Strategies for Emission Monitoring in Offshore Renewable Energy

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Offshore wind farms (OWFs) are pivotal for fulfilling the EU's goal to a sustainable energy transition and achieving climate neutrality by the year 2050, as well as the 2023 UN Climate Change Conference (COP28) agreement to triple renewable energy capacity by 2030. By 2023, a total of 75.2 GW of offshore wind capacity had been installed worldwide, with 38 GW in Chinese waters and 34 GW in European waters. Moreover, recent advancements are aiming to produce green hydrogen and secondary products such as ammonia, methanol, and synthetic fuels offshore.

The poster will showcase the developed monitoring approach for metal emissions from galvanic anodes used for corrosion protection of offshore structures: from the identification of metal tracers through analysis of these galvanic anodes to the development of analytical methods and their application to field samples. This comprehensive approach includes measurement of (tracer) metal concentrations, determination of stable metal isotope ratios, calculation of element ratios and the integration of water mass modeling to support interpretation of results. In past and current research projects, water, sediment, and biota samples from the North Sea were analyzed. Key findings from eight years of sampling will be highlighted, emphasizing trends in metal concentrations and the effectiveness of monitoring strategies. Additionally, lessons learned will be discussed to support future research and policy decisions.

This poster aims to contribute to the ongoing dialogue on sustainable offshore energy production and its environmental implications, providing valuable insights for researchers, policymakers, and industry stakeholders.

3.18.P-Mo257 Occurrence of Organophosphate Esters, Benzotriazoles, Phenols and Primary Sromatic Fiamines in Offshore Windfarm Areas from the North Sea

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The number of offshore windfarms (OWF) in Europe has been strongly increased during last years and will continue growing as a part of the efforts to reduce the CO₂ emissions. While various environmental effects have been described, the release of organic pollutants is still largely unknown. Target compounds were selected as they are used as additives or components of the polymers most frequently used in protective coatings of OWFs: polyurethane (PU) and epoxy resins (EP). Four chemical families, namely organophosphate esters (OPEs), phenolic compounds, benzotriazoles (BTRs) and derivate UV filters and primary aromatic diamines (PAAs), were selected as target compounds.

Two sampling campaigns took place in April 2024 collecting a total of 45 seawater samples from 4 different OWFs located in the Belgian and German parts of the North Sea (BPNS and GPNS respectively). Sampling design included sampling points from inside and nearby the OWF installations as well as reference areas with different anthropogenic pressures around them. Subsamples of 3 L of seawater were immediately extracted onboard by C18-polar SPE disks for the determination of phenols while for the rest of the compounds, 2 L seawater were extracted with HLB SPE cartridges. SPE sorbents were transported and eluted in the laboratory to be analyzed by LC-MS/MS.

Preliminary results from the BPNS showed the widespread presence of OPEs, with various aryl tri- and diphosphates being detected in all samples along with BTR and 4Me-BTR. The levels of both BTR and 4Me-BTR were 10 to 15 times higher in the high anthropogenic influence reference area (up to 10 ng/L). The most polluted sample was collected from the nearby area of OWF B and showed a differential pollution profile dominated by chlorinated OPEs. Triethyl phosphate (TEP) was the compound detected at the highest concentrations in most samples. Eight out of 12 analyzed phenols were detected in at least one sample and PAAs were in all cases below the limit of detection.

Even though no clear patterns have been found for this sample subset, the analysis of the remaining samples as well as further data analysis may provide more information about the sources releasing pollutants in OWF areas.

Disclaimer/Disclosure: This study was performed as part of the project Anemoi (Project number 41-2-13-22), an Interreg North Sea project co-funded by the European Union. We also acknowledge the captaincy and crew from the RV Belgica and the VWFS Atair for their collaboration on sampling campaigns.

3.18.P-Mo258 Microplastics and Offshore Wind: Context and First Data from the Belgian North Sea

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Renewable energy is a clear priority, both for the EU and for governmental and industry organizations globally. While the end of reliance on fossil fuels is indisputably a positive goal, the necessity of regulation and monitoring of the impacts of new energy generation techniques is necessary to avoid the introduction of new, unknown sources of contamination. A relevant example of developing technology is offshore energy generation, which currently exists primarily in the form of offshore wind turbines for generating electricity. Structures placed in the offshore environment require corrosion protection, including coatings in the form of paints. Recent studies have shown that a large proportion of marine particulate contaminants, or microplastics, consist of coatings that may originate from protective coatings on ships and other structures. The large coated surface area introduced to the marine environment by an offshore wind farm represents an additional source of these particles.

Seven locations in the Belgian North Sea were sampled for particulates in seawater in April 2023 as part of the Anemoi project. Samples originate from two offshore wind farms in addition to references areas with varying anthropogenic stressors. Particles were obtained by use of a filtration cascade attached to the ship seawater system with filter sizes of 300 µm, 100 µm, and 10 µm. A minimum of 1 m³ for the smallest size fraction was sampled per location. Preparation of the samples following reduction of sample volume was conducted via a two-step digestion and density separation with ZnCl₂. Analysis with laser direct infrared (LDIR) imaging constitutes the first analytical step. Further analyses and a larger dataset over two years are expected to result from work currently in progress within Anemoi.

The projected increase in offshore energy generation and marine area reserved for large structures speaks to a need for understanding and monitoring of the various types of contaminants that can be released from these structures. Currently, the behavior of particulate, or coating, particles originating from offshore wind farms is virtually unknown. Furthermore, the reliability of data concerning paint particles in the marine

environment requires improvement. This and other knowledge gaps highlight the need for the Anemoi project and further related research into emissions from new technologies associated with renewable energy, with the goal of minimizing unwanted side effects.

Disclaimer/Disclosure: This study was performed as part of the project Anemoi (Project number 41 -2-13-22), an Interreg North Sea project co-funded by the European Union. We acknowledge the crew of the RV Belgica for their collaboration.

3.18.P-Mo259 Ecological Impacts of Wind Turbine Blade Erosion on Blue Mussels (*Mytilus edulis*)

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Offshore wind farms (OWFs) introduce novel anthropogenic pressures to marine ecosystems, including the release of organic and inorganic particles from turbine blade erosion, which may impact marine life. This study investigated the potential effects of these particles and their additives on the metabolic profile of the blue mussel (*Mytilus edulis*) using ¹H-NMR spectroscopy. Mussels were exposed in the laboratory to microplastic (MP) particles derived from cryo-milled rotor blade coatings and core materials (glass fiber polymer, GFP) under a simulated worst-case scenario of extensive blade erosion. Enrichment factors (EFs) for metals and metalloids (e.g., Ba, Cu, Cd, Cr, Ni) ranged from 0.93 to 6.1, indicating a potential chemical load from the rotor blade materials. Untargeted metabolic profiling of mussel tissues revealed no significant disruptions to metabolic pathways, however trends in metabolite levels suggested potential short-term effects on neuroendocrine systems and possible long-term impacts on energy metabolism. Further research is essential to comprehensively evaluate the potential impacts of OWF erosion on marine environments, particularly in light of the planned expansion of wind farm construction as part of the EU-wide energy transition.

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3.18.P-Mo260 Analysis of coating particles from offshore wind farms in sediment samples

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Offshore energy production has risen sharply in the last decades. Consequently, the number of offshore wind farms (OWFs) is growing and chemicals may be released by the construction and operation of these structures. However, resulting impacts on the marine environment are still largely unknown. Within the EU Interreg project Anemoi, chemical emissions from OWFs and their effects are investigated. One source of emission is the particulate release of corrosion protection coatings and paints, as well as leading edge erosion of rotor blades leading to an introduction of microplastic particles into the marine environment.

Currently, only a small number of studies have investigated these paint and coating particles in marine samples. One reason for this are methodical challenges to analyse these particles in environmental samples and therefore, missing adapted protocols.

We tested different methodical approaches for separating paint and coating particles from OWFs from the sample matrix. On the one hand, different digestion protocols were compared. On the other hand, the density separation to separate the particles of interest from the sediment was adapted and improved. To do so, sediment (sandy or silty) was spiked with coating particles derived from six coatings commonly used on offshore wind turbines (four epoxy-based and two polyurethane-based) from two manufacturers. Two salt solutions with different densities were used and recovery rates calculated. Furthermore, procedures for the identification of particles by FTIR (ATR and FPA) and Pyrolysis GC/MS were optimized for the analysis of paint and coating particles and both approaches were compared. Finally, environmental samples from the North Sea were analysed using the developed protocol.

Tests showed that digestion chemicals have to be chosen with caution not to cause any harm to the paint

and coating particles. With respect to the density separation, the solution with higher density resulted in higher recovery rates for paint and coating particles. The optimization of identification methods especially included the development of an adapted library for FTIR spectra of paint and coating particles. The adapted protocol was successfully applied to a first sample set of North Sea sediment samples.

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3.18.P-Mo261 Settling velocities of particle emissions from offshore wind farms

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The technology of offshore wind is undergoing a period of rapid growth, with an enormous expansion of installed capacity expected in the coming decades. To protect offshore wind turbines against corrosion, the structures are coated with organic coatings, which deteriorate over time and subsequently detach, either through corrosion or by accidental damage during operation. The environmental impact of these detaching coatings is not investigated yet. In order to address this knowledge gap, this work focuses on the investigation of the transport of coating particles through the water column by investigating the terminal settling velocity of particles manufactured from common coating materials. Particles from epoxy and polyurethane were made as single and double-layered particles with dimensions ranging from 1 to 3 cm in flat, regular shapes such as disks and squared plates. The particles were released into an acrylic glass tank in order to determine their terminal settling velocity, using both fresh and saltwater. The double-layered particles exhibited the highest settling velocity, followed by epoxy particles, and polyurethane particles, which demonstrated the lowest settling velocity. The experiments at different salinities showed that salt water as surrounding fluid led to a decrease in settling velocities. The findings of this study represent a significant initial step towards a more comprehensive understanding of the environmental transport of corrosion protection coatings and can inform the development of strategies to mitigate adverse environmental effects associated with all types of coated marine artificial structures.

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3.18.P-Mo262 Assessing the Environmental Risks of Offshore Hydrogen Production using Energy from Offshore Wind Farm: An Anticipatory Approach

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In the context of the extensive deployment of offshore wind farms (OWF), hydrogen (H₂) is emerging as a promising energy vector to enhance electricity production from Offshore Renewable Energies (ORE). In this context, the OPHARM2 project aims to advance the knowledge on renewable H₂ production from OWF through a transdisciplinary approach including the study of energy networks integration, technology aspects and the assessment of environmental impacts. A key objective of this last point is to identify and characterise the different environmental pressures specifically generated by the development of H₂ production within an OWF.

In addition to potentially intensifying existing environmental pressures already generated by OWF (e.g. electromagnetic emissions, noise emissions, chemical pollution), offshore H₂ production could introduce new environmental issues, leading to an increase in some accidental and chronic risks to the surrounding ecosystem. A major environmental concern is the discharge of produced waters which is formed by the brine - the byproduct from desalination required to produce freshwater for electrolysis- and the cooling waters resulting from heat exchangers. For gigawatt scale OWF, discharges could exceed 10⁴m³ day⁻¹ in certain scenarios. Such chronic discharges will lead to increased salinity and temperature on a local scale. In addition, the main accidental risks involve the potential explosion of stored H₂ and substantial spills of chemicals used for desalination.

This work highlights the critical lack of empirical data and scientific resources regarding some environmental pressures, which severely hampers our ability to anticipate the potential impacts of offshore H₂ production. To address these limitations, the OPHARM2 project has undertaken a range of experimental approaches. These include the physico-chemical and ecophysiological characterization of produced water, alongside hydrodynamic modeling of plume diffusion. Collectively, these research

efforts, by advancing our understanding, aim to develop procedures for implementing a relevant Avoid, Reduce, Compensate (ARC) framework, thereby enhancing the environmental integration of offshore H2 production.

3.18.P-Mo263 Microbial Assays for Risk Assessment to Evaluate Toxicity of Chemicals and Environmental Samples

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Benefits of microbial bioassays

Bacteria are good candidates for ecotoxicity testing because of number of factors that include small size, rapid growth rate and ease of handling. The best-known tests make use of the naturally luminescent bacteria *Aliivibrio fischeri* (previously known as *Vibrio fischeri*). These tests are based on the rationale that exposure to toxins disrupts the bacterial respiratory processes, which in turn results in a reduction in light output. Reduction in light output is a relatively simple response to measure, which can then be calibrated to convey toxicity of the test sample.

MARA and LumiMARA bioassays with the incorporation of a genetically diverse multiple strains combination have significantly advanced microbial assays from single species utilisation.

Bacterial testing is generally based around assessment of the acute toxicity response of a single marine species. In contrast, testing using both MARA and LumiMARA gives a measure of acute and chronic toxicity, encompassing marine, freshwater and terrestrial species of prokaryotic microorganisms (bacteria) and additionally eukaryotic single-celled microorganism (yeast).

MARA: The MARA test assesses toxicity by measuring the growth of ten bacterial species and one yeast, following 18 hours of incubation in the presence of a concentration gradient of the test sample. As a eukaryote inclusion of a yeast species gives a valuable additional dimension to test results.

LumiMARA: LumiMARA assesses toxicity on the basis of the reduction in luminescence of 11 species of bacteria following exposure to a concentration gradient of the test sample. The test includes nine marine and two freshwater species from a variety of habitats. Two of the strains in the array are of *A. fischeri*, one of which is an equivalent to NRRL B-11177 referenced in ISO 11348-3 (Water quality Determination of the inhibitory effect of water samples on light emission of *A. fischeri* (Luminescent bacteria test) Part 3: Method using freeze-dried bacteria).

Application in the oil and gas industry: The Oslo and Paris Commission (OSPAR) Recommendation 2012/5 requires contracting parties, of which the UK is one, to implement a risk-based approach for the management of produced water from offshore oil and gas installations. MARA and LumiMARA bioassays are recommended as part of the UK's Department for Energy Security and Net Zero (DESNZ) preferred approach to achieving this.

3.18.P-Mo264 Possible Ecotoxicological Effects of Wind Turbines

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Due to problems we are facing as a result of climate change, important work is ongoing to replace fossil fuels with alternative renewable energy sources, such as wind energy and wind turbines. Besides providing new energy, offshore wind farms have been shown to have potentially positive effects in terms of reef and reserve effects and protecting them from trawling and fishing. Positive ecological effects have also been observed in terrestrial ecosystems. However, impact analyses for many energy sources are still lacking, including wind power. There is a growing concern that wind farms may contribute to an increased pollution due to leakage of particles eroding from the blades. In addition, it has been shown that the turbines also leak different kinds of oils such as hydraulic and gear oils. The aim of this study was to investigate possible ecotoxicological effects of wind turbines.

Particles were collected from moss, water and sediment from lakes adjacent to wind turbine areas as well as from control lakes for particle analysis. To get an indication of possible ecotoxicological effects, perch from lakes in these areas were collected and samples taken for gene expression and histopathological studies. In addition, turbine blades were milled for controlled trials in aquariums with cultured perch. Analyses of particle characterization and chemical content in collected particles is ongoing but particles have been found in environmental samples. Exposure to particles in controlled laboratory experiments had limited effects on the gene expression analyzed in perch but showed effects of thyroid metabolism genes. Similar results were observed in the field collected fish that also displayed unusual fat incorporation in the livers, further analyzed with histopathology.

In order to assess negative effects of wind mill relevant oils, the water accommodated fraction (WAF) of a hydraulic and a gear oil were tested on several relevant models. We exposed fish liver cells (in vitro cell line), zebrafish embryos (FET test) and copepods to a concentration range of both WAFs. Both WAFs showed toxicity with the gear oil derived WAF being much more potent leading to 100% mortality of copepods also when diluted to 0,1% and zebrafish embryos exposed to 5% of the WAF. Results show that particles and oils potentially leaking from wind turbines might have negative effects on the ecosystem. This information will hopefully aid the industry to invest in more sustainable materials.

3.18.P-Mo265 Applying Frameworks for Risk Assessment of Hydrocarbon Streams and Feeds to Alternative Non Crude Oil-Based Feeds

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Hazard and risk assessment of complex petroleum-derived substances has been in a state of continuous improvement since the 1970s, with the development of approaches that continue to be applied and refined. Alternative feeds are defined here as those coming into a refinery or chemical plant that are not hydrocarbons from oil and gas extraction such as biologically derived oils, pyrolysis oil from biomass or other, and recycled materials. These feeds are increasingly being used for production of liquid hydrocarbon streams, and hence, there is a need to assess these alternatives, subsequent manufacturing and refining processes and end products for potential risk to humans and the environment. Here we propose a tiered, problem formulation-driven framework for assessing the safety of hydrocarbon streams and products derived from alternative feedstocks in use. The scope of this work is only focused on petrochemical safety assessment, though the principles may be applicable to other chemistries. The framework integrates combinations of analytical chemistry, in silico and in vitro tools, and targeted testing together with conservative assumptions/approaches to leverage existing health, environmental, and exposure data, where applicable. The framework enables the identification of scenarios where new hazard and/or exposure assessments may be needed and incorporates tiered approaches to do so. It can be applied to enable decisions efficiently and transparently and can encompass a wide range of compositional space in both feedstocks and finished products, with the objective of ensuring safety in manufacturing and use.

3.19.A Legacy and Emerging Organic Contaminants in the Global Ocean and Polar Regions: Long-Range Transport, Local Sources and Climate Change Impacts

3.19.A.T-01 Local Sources vs Long-Range Environmental Transport of Contaminants to the Arctic – State of Knowledge, Conclusions and Recommendations

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Persistent organic pollutants (POPs) have predominantly reached the Arctic via atmospheric long-range environmental transport (LRET) from more southerly latitudes. Riverine and oceanic LRET have played a role for some more water-soluble POPs. New chemicals of emerging Arctic concern (CEACs) may also undergo LRET, but as many are consumer and industrial chemicals, they may also have local sources in the Arctic. LRET to remote monitoring sites in the Arctic is one criterion for listing chemicals on the Stockholm Convention for POPs. It is thus important to determine if Arctic measurements reflect local sources of POPs and CEACs or LRET. An assessment of local sources versus LRET of POPs and CEACs in the Arctic was carried out by members of the Arctic Monitoring and Assessment Programme (AMAP) POPs expert group and is being published as reviews and case studies in a special issue of *Environmental Science: Advances*. Results show that an expanding range of CEACs are present in the Arctic. The atmosphere is still the predominant pathway for LRET, but many CEACs are more water-soluble, thus riverine and oceanic LRET are becoming more important as pathways. Numerous local sources of POPs and CEACs to air and aquatic systems were identified, mainly linked to public, industrial and military (active and former) infrastructure, including Arctic settlements. Important local sources are municipal, industrial and military solid waste sites, untreated sewage and wastewater, open burning of solid waste, airports, active industrial and active and inactive military sites. Wildfires within the Arctic are now major emission sources of some POPs compared to LRET. LRET is still the main source of POPs and CEACs to the Arctic, but local sources may become more important considering projected Arctic climate change scenarios. Melting glaciers, ice and snow due to climate change are releasing previously deposited POPs

and CEACs that have undergone LRET. Climate change will increase wildfires, permafrost thaw and extreme weather, leading to future uncontrolled releases of POPs and CEACs from thousands of waste sites. Climate change will increase human economic activities in the Arctic including fisheries, tourism, and shipping potentially leading to increasing local CEAC emissions. Most CEACs with local emissions in the Arctic likely fall outside the Stockholm Convention, and other regulatory instruments do not typically include Arctic vulnerability in chemical risk assessments.

3.19.A.T-02 Arctic Amphipods as Bioindicators of Plastic Pollution: Identification and Simultaneous Quantification of Small Microplastics and Microlitter (< 100 µm)

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The study aimed to identify and simultaneously quantify the body burden in Arctic Amphipods (Amphipoda, Crustacea) of small microplastics, plastic additives and other microlitter components, focusing on the particles below 100 µm. These are the sizes that can be easily ingested by the organisms, hence entering the trophic web and reaching its upper layers; indeed, these are also the particles sizes that can be easily long-range transported. As part of the Microtracer project (Small MICROplastics (<100 µm) bioindicators in the changing Arctic Environment), during the summer of 2022, different genera of amphipods were collected at different sites of the Kongsfjorden, the Svalbard Archipelago. Organisms were pseudo-digested to extract microplastics and microlitter components, which were then analyzed via Micro-FTIR for characterization and quantification. Preliminary results showed no differences in the abundance of microplastics and microlitter components between the sampled sites or genera collected. The most abundant polymers and components were Polypropylene (PP) and Poly-N-Methylacrylamide (PnMA). Markers of tire wear and road particles were frequently detected, along with vulcanizing agents, specifically used in tires. According to the size distribution of microplastics and other microlitter, most particles were smaller than 100 µm. An exception is the site close to the village, where most of the particles exceed the 80-100 µm. This site might be a local source of particles. Analyzing the aspect ratio (AR), the shape of MPs and MLCs were mainly spheroidal or cylindrical. Smaller shapes and dimensions are the most common, however, due to the limits of sampling and analytical methods, these smaller particles are often overlooked. These findings highlight that amphipods can be employed as bioindicators of plastic pollution and that further studies on smaller microplastics and microlitter components are needed.

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3.19.A.T-03 Non-Target Screening of Chemicals of Emerging Concern in Marine Mammals in the Nordic Environment

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An increasing of chemicals of emerging concern (CECs) such as pharmaceuticals, personal care products, industrial chemicals, pesticides, and per- and polyfluoroalkyl substances (PFAS) are detected in aquatic ecosystems, posing significant risks to marine organisms and ecosystems. In the Nordic environment, marine mammals like harbor porpoises (*Phocoena phocoena*) and ringed seals (*Pusa hispida*) can accumulate CECs through trophic transfer. This study employs non-target screening (NTS) using high-resolution mass spectrometry (HRMS) to investigate the presence and distribution of CECs in livers of 19 individuals of harbor porpoises and ringed seals from Greenland and the Baltic Sea. All samples were extracted twice, using optimized methods for GC and LC analysis, respectively. The samples were then analyzed using gas chromatography (GC) and liquid chromatography (LC) with HRMS, to expand the chemical domain. The GC- and LC-HRMS data analysis was conducted using two different workflows on

Compound Discoverer 3.3 with both open-source and in-house libraries. A total of 108 compounds from diverse chemical classes were identified, indicating substantial exposure of these animals to a mixture of pollutants. The majority of the tentatively identified contaminants were pharmaceuticals, personal care products, pesticides, plasticizers, PFAS, and industrial additives. The detected compounds will undergo further evaluation for persistence, bioaccumulation, mobility, and toxicity through experimental or predictive approaches. Comparative analysis of chemical patterns across different species and locations will provide insights into spatial and ecological variability. The findings highlight the urgent need to prioritize identified CECs with persistent, bioaccumulative, mobile, and toxic properties for regulatory risk assessment and improved chemical management strategies in the Nordic region. This study demonstrates the utility of NTS in uncovering chemical pollution profiles and their implications for ecosystem health and conservation in marine environments.

3.19.A.T-04 Contaminant Incorporation in Sea Ice: Understanding the Roles of Sea Ice Formation and Contaminant Properties

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Despite being remote, polar seawater is widely contaminated by anthropogenic contaminants. Besides re-distributing materials during the formation, drift, and melting of sea ice, this could also put the polar food web at high risk of exposure since the porosity of sea ice, generated by brine channels, is an important habitat for microalgae. To understand how contaminants of different size (dissolved, colloidal, particulate) and composition (natural, incidental, plastic) interact, we developed a novel laboratory experimental set-up which mimics sea ice growth either as frazil or columnar ice. The morphology of both types of ice (assessed by μ -computed tomography) and its salinity were similar to that of young natural ice. Using this set-up we quantified a model dissolved species (Rose Bengal), two colloids (nanoplastic and nanosoot) and four particulate species (microplastics with two different sizes and densities) within the bulk ice (in the case of the frazil ice study) or ice and brine (separately for the columnar ice study) and underlying liquid separately. We anticipated the extent of particle incorporation into sea ice would be dictated by contaminants size and, in the case of particles, their density. During frazil ice formation, rising ice crystals may scavenge particles as they rise. This was only evidenced for microplastics, where they were 3 times enriched in bulk ice compared to underlying seawater. Nanoplastics were depleted from the bulk ice in the same proportion as sea salts. In columnar ice experiments, salts and dissolved contaminants were equally enriched in the brine channels and depleted from ice. Nanoplastics and nanosoot were also depleted from the ice, but had lower enrichment in the brine, perhaps due to their aggregation state. Microplastics followed divergent transport pathways depending on their density. High-density plastics were depleted in both brine and ice whereas low-density plastics were slightly depleted from brine and enriched in ice. Collectively, depending on both ice formation pathway and contaminant physicochemical properties, divergent incorporation could be seen. Frazil ice is responsible for the enrichment of high-density particulate contaminants in ice, whereas columnar ice is not. Both frazil and columnar ice, colloidal species are depleted from the bulk ice. This work elucidates which type of contaminants organisms may be exposed to and the fate processes which distribute them throughout the environment.

3.19.A.T-05 Investigating Climate Change Impacts on PCB-153 Exposure in Arctic Food Webs Using the Nested Exposure Model

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Global climate change is drastically changing the Arctic environment and its ecosystems. At the same time, Arctic wildlife is still exposed to high concentrations of persistent organic pollutants (POPs). A mechanistic understanding of how global POP emissions and co-occurring climate change impacts wildlife is vital to enable scientifically sound mitigation strategies. Here, we further develop and evaluate the Nested Exposure Model (NEM), a global dynamic and spatially resolved environmental fate and bioaccumulation model. NEM is applied to estimate the impact of combined changes in global contaminant emissions and climate parameters on PCB-153 exposure in the Arctic environment and

ecosystems, including polar bears (*Ursus maritimus*). NEM has previously been evaluated for selected key species in Norwegian Arctic food webs, including fish, ringed seal (*Pusa hispida*) and seabirds. Here, three new species are added to NEM; lichen (*Cladina rangiferina*), reindeer (*Rangifer tarandus*), and polar bear. The polar bear is parameterised based on literature and field data from Svalbard. NEM is run based on global emissions for PCB-153 from 1930 until 2025, estimating PCB-153 concentrations in biota in the European Arctic areas. Model estimates are compared to existing PCB-153 measurement data, supplemented with new measurements in samples of subcutaneous fat from Svalbard reindeer (n = 16). NEM estimates PCB-153 concentrations in lichen, male reindeer, and female reindeer that are a factor of 0.3, 2.3 and 6.9, respectively, compared to the measured concentrations. Estimated concentrations in individual female polar bears from 1991 to 2020 (n = 460) are a factor of 0.2–21.8 (median 1.8) compared to measured concentrations, and the temporal trend is well reproduced. The overall model bias for individual predictions of PCB-153 in female polar bears is also 1.8. This suggests that NEM performs well in reproducing concentrations in lichen, reindeer and polar bears on Svalbard, when run based on global emissions. More detailed results with a particular focus on polar bears, including exploration of selected model scenarios based on a changing climate, will be presented. NEM has potential to be expanded to other contaminants, species, and ecosystems of interest, making it a useful tool for both scientific and regulatory communities interested in understanding and protecting ecosystem and human health from legacy and emerging organic contaminants.

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3.19.B Legacy and Emerging Organic Contaminants in the Global Ocean and Polar Regions: Long-Range Transport, Local Sources and Climate Change Impacts

3.19.B.T-01 Emerging and Legacy Per- and Polyfluoroalkyl Substances in the Global Ocean and Polar Regions

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Per- and polyfluoroalkyl substances (PFASs) have been extensively studied across various environmental compartments in the global ocean. Current research reveals that PFASs are ubiquitous, earning their reputation as "forever chemicals" even in the remote polar environments. Long-range environmental transport via atmospheric circulation and oceanic currents are key pathways through which PFASs are globally distributed. In seawater and snow, perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and short-chain perfluorinated alkyl acids (C4–C6 PFAAs) are the predominant compounds, while perfluoroundecanoic acid (PFUnDA), perfluorononanoic acid (PFNA) and PFOS dominate in polar biota. Emerging PFASs, such as hexafluoropropylene oxide dimer acid (HFPO-DA) and fluorotelomer sulfonates (6:2 and 8:2 FTS), have also been detected, despite their relatively recent use as replacements for legacy PFASs. Spatial distributions suggest declining PFAS concentrations with increasing distance from continental sources, indicating ongoing discharge and transport processes. Neutral PFASs favor atmospheric transport and can transform into ionic forms through photochemical and biodegradation processes, which can be an important source of ionic PFASs in the remote ocean and polar regions. Elevated PFAS concentrations in Arctic and Antarctic coastal areas highlight the role of glacier melt and sea ice retreat as secondary sources. Given the impacts of climate change, future research should integrate observations and models to better understand PFAS biogeochemical cycles in polar regions.

3.19.B.T-02 Role of Marine Bacteria on the Interactions Between Perfluoroalkyl Substances and Bioplastics

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Recent studies on persistent organic pollutants (POPs) have revealed the presence of per- and polyfluoroalkyl substances (PFAS) in Southern Ocean environments. While the role of the marine biological pump in degrading some POPs is well-documented, PFAS are notably persistent in the environment. However, recent evidence suggests that marine bacteria may facilitate PFOS biodegradation, potentially through desulfurization, as indicated by transcriptomic analyses of bacterial communities in PFOS-spiked waters. Additionally, the increasing presence of bio-based biodegradable plastics (e.g., PLA)

in marine environments raises concerns, as their degradation rates are significantly slower under marine and polar conditions compared to compost environments, complicating their environmental impact. To elucidate PFAS biodegradation mechanisms, the reproducibility of proposed pathways and factors influencing the process must be studied. This includes co-metabolic strategies in marine bacteria under sulfur-enriched conditions, such as those in Port Foster Bay (Deception Island, Antarctica). The role of PLA bag fragments in PFAS adsorption/desorption, their leaching of plastic additives, and their potential as carbon sources for biofilm-forming bacteria are also critical considerations. These factors may significantly affect bacterial communities involved in PFAS degradation and their functional mechanisms. An experiment was conducted during the Spanish Antarctic Campaign (February 2024) using seawater collected from Port Foster Bay at 40 m depth. Diatoms, abundant in Antarctic waters, were filtered out for half of the treatments. Seawater with and without diatoms was spiked with a sulfonated PFAS mixture under abiotic and biotic conditions, with and without PLA bag fragments. Incubations were maintained under natural sunlight and temperature in sterile, ultra-clean PP bottles for 8 days. Sampling at various time points included analyses of PFAS chemical degradation, chlorophyll-a levels, nutrient availability, bacterial abundance, community composition, and transcriptomic profiles. This study aims to deepen understanding of PFAS interactions with Antarctic bacteria, highlighting how environmental factors, such as phytoplankton presence and their by-products, along with other pollutants, influence the degradation of these persistent "forever chemicals" known for their bioaccumulation and toxicity.

3.19.B.T-03 Emerging Organic Compounds Occurrence in the “Touristic” Antarctic Peninsula

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The ecosystems of the polar regions are facing actual changes stemming from both natural phenomena and human activities. Antarctica is now impacted not only by persistent pollutants generated in inhabited continents arriving via long range transport, but also by local sources including scientific bases and an increasing touristic pressure. Indeed, in the last years, daily use chemicals including non-persistent ones such as pharmaceuticals, personal care products or similar polar Contaminants of Emerging Concern (CECs) have been measured in the Antarctic Peninsula surroundings. The aim of this study was to develop and implement an advanced, wide-ranging analytical method by means of solid-phase extraction (SPE) followed by analysis with high-resolution mass spectrometry (LC-HRMS, qOrbitrap). This methodology was designed to detect and quantify a variety of organic pollutants in snow and water samples collected in November 2023 from the coastal Antarctic Peninsula. The families of compounds analysed in this study include endogenous hormones, pesticides, industrial chemicals, lifestyle products, and pharmaceuticals. Briefly, clean and penguin colonies snow, surface and subsurface seawater and on-board residual water from a touristic boat were gathered in November 2023 in the coastal Antarctic Peninsula. Between 200 mL and 2 L of water or melted snow were filtered onto GF/F glass filters on board before its extraction with HLB Oasis cartridges using a slightly modified, in-house-validated protocol. Cartridges were shipped to the University of the Basque Country frozen, eluted and concentrated prior injection. The instrumental analysis was performed on a Thermo Scientific Dionex UltiMate 3000 UHPLC coupled to a Thermo Scientific Q Exactive Focus quadrupole-Orbitrap equipped with a heated ESI source (Thermo, CA, USA). The injection of the extracts was performed in positive and negative ionization modes in full scan data-dependent MS2 discovery acquisition. A selection of around 300 compounds, including many different CECs families was used for a target analysis.

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3.19.B.T-04 High-Resolution Record of Global Background Persistent Organic Pollutants from Antarctic Marine Sediments

Jun-Tae Kim, Korea Institute of Science and Technology, "Korea, Republic of"

Introduction:

The global spread of persistent organic pollutants (POPs) serves as a key marker of human impact in the Anthropocene. Antarctic marine sediments, particularly diatom ooze with high sedimentation rates, offer unique opportunities to reconstruct historical pollution trends. However, the potential of these sediments

as archives of atmospheric deposition remains largely unexplored.

Materials and Methods: A 67.5 cm marine sediment core was collected from the Antarctic Peninsula's continental shelf at 730m depth. The core chronology was established using ²¹⁰Pb dating. Multiple pollutant classes including PCBs, PBDEs, OCPs, PAHs, and OPAHs were analyzed using GC-HRMS and GC-MS/MS. Geochemical proxies (TC, TN, TOC) were measured to characterize organic matter content and origin. The observed deposition patterns were compared with MPI-MCTM modeling predictions.

Results and Discussion: The core provided a continuous 68-year record with a high sedimentation rate (0.99 cm/yr) and no stratigraphic disturbance. Geochemical analyses revealed high organic content (TOC: 0.926-2.07%) with consistent marine origin (OC/TN: 6.84-8.58). Temporal trends in POPs concentrations matched global emission patterns, with peak levels observed in the mid-1990s for most compounds. Notable findings included recent rebounds in DDTs, BDE 209, and HCHs concentrations post-2010, potentially linked to glacial melting and increasing primary production.

Conclusions: This study presents the highest-resolution record of atmospheric pollutant deposition in the Antarctic Ocean during the Anthropocene. The diatom ooze successfully preserved global emission signals, demonstrating the Southern Ocean's significant role as a sink for anthropogenic pollutants. The observed recent rebounds in certain POPs highlight the potential impacts of climate change on pollutant cycling in polar regions.

3.19.P-We261 Investigating Microplastic Transport in Western North American Snow

Aleksandra Karapetrova¹, Win Cowger², Sonya Maple³, Audrey Jeanne Braun¹, Andrew Gray¹ and Jay Gan¹, (1)University of California Riverside, United States, (2)Moore Institute for Plastic Pollution Research, United States, (3)California Department of Fish and Wildlife, United States

The atmosphere is one of the least studied environmental compartments for microplastic (MP) transport likely because of the relatively small size of airborne MPs and the challenges in identifying them. MPs from within and below clouds can deposit into alpine terrestrial environments in snowfall via atmospheric scavenging. To measure the extent of MP atmospheric pollution, 11 sites across western coastal North America were sampled and analyzed for MP presence in fresh snowfall, months-old summer surface snow, and stratified deposits in snow pits. Following this study, fresh snowfall at 7 sites in the California Sierra Nevada mountain range were regularly sampled to determine the deposition rate of MPs in the region. MPs were detected and characterized using an improved method integrating linear array μ -Fourier Transform Spectroscopy (μ FTIR) and batch spectral analysis using the open-source platform Open Specy. This method enables high-throughput detection and analysis of MPs > 50 μ m Feret diameter. The recovery rates of MPs from sample filtration to data analysis were an average of 98% for 250-300 μ m MPs and 76% for 70-90 μ m MPs. The low field and laboratory contamination rates (? 1 polyamide fragment per blank) were very low. The concentrations of MPs in the fresh snowfall of remote sites and sites proximal to MP sources ranged from 5.1-150.8 p/L and 104.5-325 p/L of meltwater, respectively. Summer surface snow that was several months old had MP concentrations ranging from 57.5-539 p/L of meltwater, and snow sampled at different depths within a snowpack had concentrations ranging from 35-914 p/L. All particles identified as MPs were fragments, and most (~ 90%) were under 200 μ m. The most common polymer types found were polyamide, polyethylene terephthalate, polystyrene, polyolefin, and polyvinylester polymers. These are the first field observations of MP in the alpine snow in the Cascade Range, Sierra Nevada Mountains, and arctic tundra snow on the Seward Peninsula, USA. An atmospheric model of MPs based on GEOS-Chem was used to understand potential sources of MPs in the California Sierra Nevada mountain range. Our results also demonstrate the value of a streamlined method that may be used for measuring MPs in the environment, contributing to a better understanding of long-range MP transport, and the global extent of MP pollution.

3.19.P-We271 Microplastics Distribution and Occurrence in Greenlandic Fjords

Tristan Zimmermann¹, Lars Michael Hildebrandt², Cornelius Vetter³, Claudia Elena Schmidt³, Daniel Profrock¹ and Helmuth Thomas³, (1)Inorganic Environmental Chemistry, Helmholtz-Zentrum Hereon, Germany, (2)Department of Inorganic Environmental Chemistry, Helmholtz-Zentrum Hereon, Germany, (3)Helmholtz-Zentrum Hereon, Germany

The northeastern coast of Greenland (Tunu) is hardly, if at all, inhabited and reveals near pristine characteristics. As sea ice still covers the fjords and ocean for most of the year, the population and vessel densities are close to zero. Nevertheless, microplastics (MPs, 1 5,000 μ m) and nanoplastics (< 1 μ m) are to be found even in places without nearby pollution sources. To date, there is only a limited number of studies on micro- and nanoplastics (MNPs) in the Arctic and numerous questions regarding their transport, distribution, fate, and final consequences for this unique environment remain unanswered.

Within this context microplastic sampling was conducted at eight locations within the fjords of East Greenland and the Denmark Strait with the research vessel Maria S. Merian in August 2022 (cruise

MSM110, Ecological tipping cascades in the Arctic seas (ECOTIP)), using the Geesthacht Inert MP Fractionator. Four fractions were taken per sampling station: one hydrocyclone and three filter cartridges with a 300 µm, 100 µm and 10 µm mesh size. 2.5 to 5.2 m³ of ocean water were filtered per sample to process a representative water volume. Transflection-mode infrared spectroscopic measurements were conducted using an Agilent 8700 Laser Direct Infrared (LDIR) Chemical Imaging System.

Measured MP number concentrations ranged between 12 ± 4 L⁻¹ (1 SD, n = 4) at the mouth of the Scoresby Sound and 1.0 ± 0.8 L⁻¹ (1 SD, n = 3) in the Dove Bay. Twelve polymer types were identified in total. PET is by far the most abundant polymer type in all 18 samples with an overall average share of $96 \pm 3\%$ (1 SD, n = 18). Polylactic acid (PLA) is the second most frequent polymer type with an average share of $1.6 \pm 0.5\%$ (1 SD, n = 18).

The fjords of East Greenland and the Denmark Strait are notable hotspots for MP pollution. On average, PET constituted 96% of the total MP abundance, while PLA constituted 1.6%. The level of PLA detected is believed to be the highest ever recorded in marine samples, which raises questions regarding its source and transport. The results revealed no discernible trends between MP concentration, polymer composition, or size distribution and glacial meltwater inputs. Therefore, it is assumed that oceanic currents and sea ice transport exert a substantial influence on the distribution of MPs. The collection of replicate samples proved to be important in demonstrating the temporally varying patchiness of MP distributions.

3.19.P-We278 Unraveling the Effects of Microfibers Across Treatment Stages via a Critical Prey Species, in the Context of Climate Change

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Mysid shrimp are an important prey item in marine and freshwater food webs globally, including those containing key predators such as gray whales and salmonids. Gray whales require millions of zooplankton daily, primarily mysids, and are threatened by worsening climate conditions such as ocean acidification. Additionally, whales can consume over 20 million microparticles (primarily microfibers) daily from mysid prey. Microfibers exert chemical and physical toxicity, but knowledge is sparse regarding how much each contributes to organismal stress. Therefore, our goals were to further estimate impacts to coastal food webs and to better understand the contribution of chemical vs. physical effects from microfibers, in addition to understanding impacts in the context of climate change on these critical prey species. We quantified responses to polyester microfibers across treatment stages (grunge/naïve, ozonated, chemically modified) and weathering states, including low pH exposure, on native and surrogate mysids. Briefly, microfibers were produced from textiles across treatment stages provided by an anonymous clothing manufacturer. Mysids were exposed to 5 (*A. bahia*, purchased) or 10 microfibers (*H. sculpta*, native field collected) / mL for 7 days or 48 hours, respectively. Following exposures, *A. bahia* underwent a sublethal low pH challenge. Behavioral toxicity was measured in both species. Results demonstrate that growth was reduced in *A. bahia*, and behavioural responses are predominately hypoactive, being most sensitive to chemical treatment or weathering, and potentially exacerbated at a lower pH. Behavioural impacts are more pronounced in native *H. sculpta*. These effects have broad implications for individual, population and ecosystem health. Current studies investigate the same fabric treatments in cotton in both species, including RNA seq analysis, and seek to better understand microfiber occurrence in relevant coastal areas. To our knowledge, this is one of the first studies utilizing textile microfibers across known treatment stages in both native and surrogate (model) species and could directly inform more sustainable textile production in the future.

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3.19.P Legacy and Emerging Organic Contaminants in the Global Ocean and Polar Regions: Long-Range Transport, Local Sources and Climate Change Impacts

3.19.P-We259 Tissue Distribution of Chlorinated Paraffins in Finless Porpoises (*Neophocaena phocaenoides*): Risk Assessment via Physiologically Based Toxicokinetic (PBTK) Model

Yetong Shao, Yuefei Ruan and Qi Wang, City University of Hong Kong, Hong Kong (Greater China)
Chlorinated paraffins (CPs) are high-production chemical mixtures that accumulate significantly in marine

animals. With the phasing-out of short-chain CPs (SCCPs) since 2024 in China, environmental levels of medium-chain (MCCPs) and long-chain CPs (LCCPs) are expected to rise, leading to potentially increasing exposure risk to marine predators. However, the tissue distribution of CPs, particularly MCCPs and LCCPs, remains understudied. This study simulated the toxicokinetics and tissue distribution of SCCPs, MCCPs, and LCCPs in finless porpoises (*Neophocaena phocaenoides*) using a physiologically based toxicokinetic (PBTK) model. The model was optimized through calibration and validation with bioaccumulation data of CPs in different tissues (liver, kidney, brain, muscle, and blubber) of stranded finless porpoises sampled in Hong Kong waters. Results showed that lipid-normalized concentrations of CPs increased with age across all tissues, due to low clearance rates. The liver had the highest concentrations, followed by muscle and blubber. Although blubber is often used in environmental monitoring, it contains lower CP levels compared to liver and muscle, suggesting potential underestimation of bioaccumulation when using blubber alone. As such, this study enhances understanding of CP bioaccumulation across different tissues over time, aiding in assessing the health impacts and ecological risk of CPs on finless porpoises.

3.19.P-We260 Antarctica Sampling and Logistic Hurdles for Cyclic Volatile Methylsiloxanes (cVMS)

Jeremy Durham¹, Debra Ann McNett¹, Mark Irvine², Isabel Sauermilch³, Rita M. Seston⁴, Reinhard Gerhards⁵, Robert Bialik⁶, Pernilla Nizzetto⁷, Dragomir Mateev⁸ and Evgen Dykyi⁹, (1)Dow Chemical Company, United States, (2)ERM - Environmental Resource Management, United Kingdom, (3)ERM - Environmental Resource Management, Germany, (4)Hyla Consulting LLC, United States, (5)Gerhard Consulting, Germany, (6)Poland Antarctic Institute, Poland, (7)NILU, Norway, (8)Bulgaria Antarctic Institute, Bulgaria, (9)Ukraine Antarctic Institute, Ukraine

Environmental monitoring of cyclic volatile methylsiloxanes (cVMS) occurred during the austral summer of Antarctica 2023-2024, with additional air samples occurring throughout the following year in select locations. The sampling locations included Polish Henryk Arctowski station, Bulgarian St. Kliment Ohridski station, Ukrainian Vernadsky station and the Norwegian Troll station.

The objective of the project is to determine the potential presence of cVMS in air, surface media, and aquatic biota in Antarctica. The study design included the sampling and analysis of air, terrestrial surface media (i.e., soil and vegetation), aquatic biota, and sediments to obtain accurate cVMS concentrations with a thorough understanding of the influence of local sources and potential bias from collection and analytical procedures. State-of-the-art sampling, quality control procedures and analytical methods were developed and used to minimize the artifacts due to sample contamination or loss and to evaluate the long-range environmental transport (LRET) and back deposition potential of cVMS in remote regions. While Antarctica is often considered to be a remote region in the context of environmental pollution resulting from anthropogenic activities, the presence of research stations and increasing pressure from tourism were considered as potential local sources in the study design.

Environmental monitoring in Antarctica and remote locations brings about many sampling and logistical challenges. This presentation will highlight the importance of a detailed and in-depth study plan and standard operating procedures (SOPs) with the focus of this presentation on 1) preparation of materials, 2) sampling and 3) sample logistics.

1) Preparation of materials includes collection documents, pre-cleaned sampling equipment and storage containers and development of sampling kits (equipment containers and quality control samples)

2) Sampling includes the detailed SOPs, documentation of sampling activities and utilization of quality control samples.

3) Sample logistics include the packing and tracking of samples via different routes and carriers and working through customs to ensure sample integrity throughout shipment to laboratory.

3.19.P-We261 Investigating Microplastic Transport in Western North American Snow

Aleksandra Karapetrova¹, Win Cowger², Sonya Maple³, Audrey Jeanne Braun¹, Andrew Gray¹ and Jay Gan¹, (1)University of California Riverside, United States, (2)Moore Institute for Plastic Pollution Research, United States, (3)California Department of Fish and Wildlife, United States

The atmosphere is one of the least studied environmental compartments for microplastic (MP) transport likely because of the relatively small size of airborne MPs and the challenges in identifying them. MPs from within and below clouds can deposit into alpine terrestrial environments in snowfall via atmospheric scavenging. To measure the extent of MP atmospheric pollution, 11 sites across western coastal North

America were sampled and analyzed for MP presence in fresh snowfall, months-old summer surface snow, and stratified deposits in snow pits. Following this study, fresh snowfall at 7 sites in the California Sierra Nevada mountain range were regularly sampled to determine the deposition rate of MPs in the region. MPs were detected and characterized using an improved method integrating linear array μ -Fourier Transform Spectroscopy (μ FTIR) and batch spectral analysis using the open-source platform Open Specy. This method enables high-throughput detection and analysis of MPs > 50 μ m Feret diameter. The recovery rates of MPs from sample filtration to data analysis were an average of 98% for 250-300 μ m MPs and 76% for 70-90 μ m MPs. The low field and laboratory contamination rates (? 1 polyamide fragment per blank) were very low. The concentrations of MPs in the fresh snowfall of remote sites and sites proximal to MP sources ranged from 5.1-150.8 p/L and 104.5-325 p/L of meltwater, respectively. Summer surface snow that was several months old had MP concentrations ranging from 57.5-539 p/L of meltwater, and snow sampled at different depths within a snowpack had concentrations ranging from 35-914 p/L. All particles identified as MPs were fragments, and most (~ 90%) were under 200 μ m. The most common polymer types found were polyamide, polyethylene terephthalate, polystyrene, polyolefin, and polyvinylester polymers. These are the first field observations of MP in the alpine snow in the Cascade Range, Sierra Nevada Mountains, and arctic tundra snow on the Seward Peninsula, USA. An atmospheric model of MPs based on GEOS-Chem was used to understand potential sources of MPs in the California Sierra Nevada mountain range. Our results also demonstrate the value of a streamlined method that may be used for measuring MPs in the environment, contributing to a better understanding of long-range MP transport, and the global extent of MP pollution.

3.19.P-We262 Patterns in Mercury Biomagnification in Boreal and Subarctic Lake Food Webs – Similarities and Differences Across Climate and Productivity Gradients

Alexander Piro¹, Natalia Kozak², Ossi Keva¹, Emmi S. Eerola¹, Katja Kulo³, Timo Ruokonen³, Jan Weckstrom¹, Tommi Malinen⁴, Mikko Kiljunen⁵, Sami Taipale⁶ and Kimmo Kalevi Kahilainen¹,
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Climate change and intensive land use are significant and increasing pressures on northern lake environments, thus transforming the composition and interactions of aquatic ecosystems. These changes will likely influence mercury (Hg) dynamics in these systems. Increasing temperatures and an extended warm season enabling increased agriculture and forestry activities jointly drive the leaching of nutrients and toxic Hg from catchments into waterbodies and potentially influencing biomagnification of Hg up through aquatic food webs to unknown extents across complex landscape gradients. How this process occurs along an extended climate-productivity gradient paired with catchment metrics has not been thoroughly explored. We sampled lake food webs from primary producers to top predators across a landscape gradient in 19 subarctic and 16 boreal lakes in Finland. We tested the joint effects of climate, productivity, and catchment metrics in a principal component analysis (PCA) on total Hg (THg) biomagnification in food webs across both regions by assessing the trophic magnification slope (TMS) and mercury baseline (baseline THg) through the regression of log10 transformed THg (log10THg) against trophic levels (TL). We then used principal components comprised of climate, productivity, and catchment variables jointly in stepwise general linear regression models to assess TMS and baseline THg. We found differing regional importance regarding influences on THg biomagnification. Subarctic lake THg biomagnification variables, TMS and baseline THg, demonstrated pronounced importance with a negative trend regarding lake productivity classification along a climate-productivity gradient (PC1), while the boreal TMS required all relevant principal components (PC1, PC2, PC3) for modelling and still achieved lesser predictive capacity compared to subarctic lakes. Principal components were not significant for the modelling of boreal baseline THg. A confluence of more diverse food webs with a higher exposure to intensive land use over a longer period in the boreal compared to the subarctic is the likely reason for the lower predictive power observed in boreal TMS and baseline THg. Ongoing climate warming and northward intensification of land use suggest that pristine subarctic lakes will likely trend towards the higher impact status seen in the boreal lakes in the future.

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would like to thank the Lammi Biological Station and Kilpisjärvi Biological Station for providing research facilities and equipment. We thank all individuals who assisted in the field and laboratory work involved in this work.

3.19.P-We263 Enantioselective Accumulation and Trophodynamics of p-Phenylenediamines Antioxidants and Their Quinones in the Mangrove Ecosystem

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p-Phenylenediamines (PPDs) and their quinones (PPDQs) are contaminants of emerging concern. However, limited information exists on their occurrence and fate in the aquatic environment, which are particularly unknown in the mangrove wetlands. Herein, the distribution, bioaccumulation, trophodynamics and chiral signatures of PPDs and PPDQs were explored in Qi ao Island Mangrove Nature Reserve of China. Concentrations of PPDs and PPDQs in mangrove biota ranged from not detected (nd) to 352 and nd to 49.4 ng/g lipid weight, respectively. Terrestrial birds had higher concentrations of PPDs than mangrove aquatic biota. The enantiomeric compositions of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) and its quinone (6PPDQ) were evaluated in the field for the first time. A significant enrichment of (+)-6PPD was observed in crab and fish, and a preferential enrichment of (-)-6PPD was found in birds. Nonracemic signatures were also found for 6PPDQ in mangrove biota, with the preference accumulation of (+)-6PPDQ. IPPD, DPPD, DTPD and DPPDQ exhibited significant trophic magnification in the mangrove food web, while no clear trophodynamics trend was found for other target PPDs and PPDQs. Further investigation is necessary to explore the factors affecting the trophodynamics of PPDs and PPDQs and the mechanisms underlying the enantioselective bioaccumulation of 6PPD and 6PPDQ.

3.19.P-We264 Do Local Sources of Pollution Influence the Exposure of Ringed Seals (*Pusa hispida*) Analyzed in Arctic Contaminants Monitoring Programs?

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The monitoring of contaminants in Arctic wildlife is mainly designed for long-range transported chemicals and supports international initiatives to mitigate global threats from chemical pollution. As human activities increase in the Arctic, the question of potential impact of local pollution on wildlife exposure has emerged. In this study, we examined the current monitoring practices and the state of knowledge of pollutants in Arctic ringed seals (*Pusa hispida*) from Canada, Greenland, Norway, Russia, and Alaska and tested the potential influence of local pollution sources from different locations in Canada (2008-2022). Multivariate constrained ordination was applied to examine the effects of ringed seal biological metrics (age, sex, girth, dietary proxies), settlement population density, and potential local contaminant sources (infrastructures, industries) on contaminant concentrations [polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), per- and polyfluoroalkyl substances (PFAS), polybrominated diphenyl ethers (PBDEs), trace elements] in seals. Results indicated that biological data, location, and local source variables explained 13% (PBDEs), 38% (OCPs), 42% (trace elements), 45% (PFAS), and 68% (PCBs) of the contamination variation in seals through time. Variation partitioning showed that local source variables (e.g., infrastructure, industries) explained less than 2% of variation for PFAS, OCPs, PBDEs and trace element compared to 17.2% for PCBs. Results suggest that effects of local contaminant sources are tightly linked to study site characteristics as well as seal biology. Local sources should be identified and assessed close to communities to ensure efficient mitigation measures.

3.19.P-We265 Recording the Baseline Before the Change: First Steps Towards an Integrated Chemical and Biological Pollution and Effects Assessment Off Dronning Maud Land Antarctica

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With rising global surface temperatures, an increasing number of volatile pollutants emitted in lower latitudes of the southern hemisphere will evaporate and undergo an, in turn, prolonged atmospheric long-range transport. Thus, more persistent organic pollutants (POPs) will eventually deposit in the southern cold-trap - the Antarctic. At the same time, a warming ocean will accelerate the re-release of ice-sealed contaminants into the Southern Ocean. Consequently, an increase in bioavailable anthropogenic pollution is a foreseeable perspective for Antarctica and its biota. However, in Dronning Maud land, this intensification of pollution stress and its biological implications will proceed somewhat unnoticed due to a

lack of holistic, baseline investigations of pollutants and their impact.

Therefore, in this project, we will perform an integrated pollution and risk assessment for Dronning Maud land and its biota, based on a three-month sampling campaign of the sea-ice of Atka Bay. Using innovative passive sampling techniques, we will investigate the current status of POP pollution in different environmental matrixes ranging from sea ice to (deceased) penguin tissue.

Starting at the onset of the melt season and extending into austral summer, we will follow up on the release of pollutants from the ice into the seawater and their putative uptake into ice alga and further into kill feeding on them. By determining pollutant body burdens, we will assess pollutants bioaccumulation and further biomagnification in Antarctic key species. Furthermore, in evaluating this in a recurring sample collection, we will learn about the seasonality of POP pollution.

As a significant counterpart to chemical analytics, we will complement the screening for POPs with a biological impact assessment. For this, we will investigate the biomarker response in krill to employing cellular and subcellular biomarkers of detoxification, oxidative stress, and regeneration. Again, the seasonal, stationary, sampling approach will enhance our knowledge of the variability of these physiological and potentially pathological responses. This will facilitate the interpretation of future sampling campaigns and forecast biomarker behavior. Involving other research stations in developing this concept, we aim to expand the current boundaries of pollution research and contribute to establishing future comprehensive and coordinated pollution and effect assessments in Antarctica.

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3.19.P-We266 PLASTFLOW - How Much Plastic Flows Into the Sea? Quantifying Plastic Fluxes and Identifying Plastic Hotspots in the Scheldt Estuary in Belgium

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Within the PLASTFLOW project, we aim to update the assessment of litter levels and plastic fluxes in the Scheldt estuary, Belgium, focusing on changes since the 2020-2021 baseline study. Through follow-up measurements during 2024-2025, the project focuses on quantifying seasonal variations, refining computational models of plastic fluxes, and providing clear insights into the amount of plastic flowing into the sea for policy development.

Sampling occurs at six locations along the Scheldt River, covering water and sediment across various depths to account for the behaviours and fluxes of different polymer types and densities. Surface water is sampled using net-based methods, while pollution in the water column is assessed with suspension and bedload samplers. Sediment is collected from the riverbed using a Van Veen grab, and riverbank samples are taken via quadrant-based approaches along transects, complemented by drone analysis. The combinations of these innovative methods provide a representative snapshot of macro-, meso-, and microlitter distribution.

During the planned campaigns within PLASTFLOW, three types of follow-up measurements are being acquired, each time during spring, summer, autumn, and winter: tidal cycle measurements, to assess and quantify the tidal movement of plastic particles along the Scheldt River; spot sampling measurements; and hotspot measurements, to assess long-term trends in microplastic deposition and identify key environmental parameters of plastic retention and accumulation.

Initial results point out Antwerp as a hotspot for microplastics in both water and sediment (286-615 plastics/m³), pellets (2822-6611 pellets/m³), mesoplastics (56-1238 plastics/m³), and macroplastics (23-181 plastics/m³). While areas further upstream appear to be hotspots for microplastics as well (151-778 plastics/m³ at Wintam and 275 plastics/m³ at Temse), meso- and macroplastic as well as pellet abundance on the riverbanks in both these areas was low (0-90 pellets/m³, 1-20 mesoplastics/m³, and 2-7 macroplastics/m³).

All acquired samples will be used to produce computational models that will be applied to determine the

transport of plastic particles along the Scheldt estuary, and to validate those models. By integrating these results into computational models, the project will enhance predictions of plastic fluxes and accumulation, supporting updates to monitoring frameworks and aligning with the EU Green Deal's Zero Pollution Action Plan.

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3.19.P-We267 Factors Influencing Regulated Pollutants in the Air of Admiralty Bay, Antarctic Peninsula

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Antarctic is an important sink for pollutants due to its low sea surface temperatures, long periods without solar radiation, and well-defined seasonality, enhancing pollutant deposition and limiting degradation. However, climate change challenges this role, as rising temperatures accelerate re-volatilization of persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs) from soil and snow. This study aimed to investigate POPs and PAHs in the atmosphere of Admiralty Bay, Antarctic Peninsula, using semi-permeable membrane devices (SPMD) to understand the factors influencing their presence in the air. The sampling devices were deployed at two locations in the austral summer of 2023, spring of 2023, and summer of 2024. Identification and quantification of target analytes were performed using an Agilent Model 7890B gas chromatograph coupled to an Agilent Model 7010B triple quadrupole TQMS operating in multiple reaction monitoring mode. The detection of high concentrations of low-chlorinated PCBs and HCB being the most prevalent pollutant - both restricted under the Stockholm Convention - suggests that long-range atmospheric transport (LRAT) is the main pathway for POPs. Trajectory models indicate that air masses often originate from the Southern Ocean, South America, and the Antarctic Peninsula and act as potential sources of contamination. Higher PAH concentrations near local activities indicate an influence from research stations and tourism emissions. The predominance of HCBs over PAHs may indicate a significant role in degradation processes. Higher levels of contaminants during the austral summer and at coastal sites may indicate potential re-volatilization from water, soil, and snow reservoirs. In summary, the distribution of POPs and PAHs in Admiralty Bay results from multiple factors, including LRAT, local emissions, physicochemical properties, secondary sources, meteorological conditions, and air mass dynamics. This study demonstrates the feasibility of using SPMDs in Antarctica, providing a practical and efficient method for continuous monitoring in remote and challenging conditions. It also contributes to understanding pollutant dynamics in the Antarctic environment, highlighting the need for global emission controls and continuous monitoring of this vulnerable region.

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3.19.P-We268 Emerging Per- and Polyfluoroalkyl Substances (PFAS) Induced Intestinal Barrier Dysfunction in Marine Medaka (*Oryzias melastigma*)

Naiyu XIE, Yuefei Ruan and Qi Wang, City University of Hong Kong, Hong Kong (Greater China) Perfluoroethylcyclohexane sulfonate (PFECHS) and 6:2 chlorinated polyfluoroalkyl ether sulfonate (6:2 Cl-PFESA) are emerging per- and polyfluoroalkyl substances (PFAS) and are considered as alternatives to perfluorooctane sulfonate due to their similarity in chemical structure and physicochemical properties. However, their adverse effects on marine organisms are not fully understood, particularly concerning intestinal health. In this study, newly fertilized marine medaka (*Oryzias melastigma*) was exposed to 6:2 Cl-PFESA and PFECHS at environmentally relevant concentrations (nominal: 0.1, 0.3, and 1.0 µg/L) for 90 days to investigate their potential digestive toxicity. Results showed that 6:2 Cl-PFESA exhibited a higher bioaccumulation potential compared to PFECHS, and both emerging PFAS preferred to accumulate in the liver rather than the intestines. Physiological analysis demonstrated that PFECHS exposure

disrupted the digestive system, as evidenced by increased activities of digestive enzyme (e.g., disaccharidase, alkaline protease, and neutral protease). Microbiome sequencing revealed that significant alterations in intestinal microbial community structure were observed in PFECHS-exposed medaka, with decreased relative abundance of beneficial bacteria (e.g., *Bacteroides* and *Pseudomonas*) and an increased proportion of *Alkalimarinus* and pathogenic bacteria *Vibrio* in the PFECHS-exposed group. Co-occurrence network analysis indicated a reduction in species interactions and network complexity after treatment with PFECHS. Correlation analysis revealed a close association between pathogenic bacteria and digestive enzymes. Our research emphasizes the potential risks of emerging PFAS and provides insights to evaluate the relationship between gut microbiota and intestinal barrier function, highlighting the necessity to establish risk assessment and management strategies for new pollutants.

3.19.P-We269 Pharmaceuticals and Other Compounds of Emerging Concern in the Wastewater Effluent of Cruise Ships in the Arctic

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The cruise ship industry is the fastest-growing industry in the tourism sector. The size, passenger capacity, and number of amenities on modern cruise ships make them comparable to terrestrial settlements. As such, they can inflict similar pressures on the surrounding environment as urban areas, such as noise, air, and water pollution. Due to improvements in ship design and technology, as well as receding polar ice, cruising in polar regions has seen an increase of 118% between 2018 and 2022.

This study investigates the chemical composition of wastewater from three expedition cruise ships operating in the North Atlantic and Arctic waters, focusing on pharmaceuticals, personal care products, and industrial chemicals. Wastewater was collected from the final holding tank prior to discharge, containing treated black-and grey water. Over 150 distinct compounds were identified or present with a high probability at Schymanski level 1 and 2. Analysis was done using high-performance liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry (HPLC-ESI-QTOF). Twenty seven compounds were identified with high probability in all three ships, which warrant priority attention in future studies. For all ships, pharmaceuticals dominated the chemical profile (43 -58% of identified compounds), followed by industrial chemicals (21-30%), and natural compounds (12-15%). The compound group of the highest concern is pharmaceuticals due to their potential for biological activity at low concentrations, even after wastewater treatment. The pharmaceuticals present were primarily cardiovascular medications. Additionally, multiple antibiotics were detected, raising concerns that cruise ship wastewater effluent could contribute to the spread of antibiotic-resistant bacteria.

Other ships, like naval vessels and cargo ships, are expected to have distinctly different chemical profiles, with industrial chemicals dominating cargo ship wastewater, while passenger-heavy ships such as ferries may have higher concentrations of pharmaceuticals and personal care products. Investigating wastewater discharge across shipping industries, particularly in sensitive habitats such as polar regions and coral reefs, is crucial to understanding the broader impact of shipping on global pollution levels.

3.19.P-We270 Nontarget Analysis of Arctic Sediments: An Empirical Indicator of Persistent Chemicals Overlooked by Regulation

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Arctic sediments function as a receptor for global anthropogenic chemicals. As a consequence, detection of chemicals in remote Arctic sediments offers empirical evidence of their persistence, long-range transport (LRT) potential, and, indirectly, high production volumes. In other words, detection of chemicals in remote Arctic sediments is useful for assessing the persistence criteria for regulation. The overarching aim of this project is to apply state-of-the-art mass spectrometric tools on high Arctic sediments to identify persistent contaminants of global concern, and then apply statistical and modeling approaches to further understand their emission sources and LRT. Three high Arctic lake sediment cores and 81 circum-Arctic shelf surface sediments were obtained from archives at Stockholm University and Environment and Climate Change Canada to assess the prevalence, temporal and spatial variability of contaminants, along

with dominant LRT pathways.

Samples were characterized using a newly developed gas chromatography-atmospheric pressure chemical ionization-ion mobility-high resolution mass spectrometry method, for simultaneous target, suspect, and non-target analyses of a wide range of hydrophobic organic chemicals. In addition to known substances (including polycyclic aromatic hydrocarbons, polychlorinated biphenyls, organochlorine pesticides, and organophosphate esters), we annotated 78 substances at Confidence Level 2/3 according to the Schymanski scale. Of these substances, 81% were reported in Arctic sediments for the first time, while 86% were not part of the Arctic Monitoring and Assessment Programme's list of chemicals of emerging Arctic concern, including a novel fluorotelomer methylsulfone. Taken together, this work offers new knowledge on emission sources and global transport of persistent chemicals of global concern while providing scientific guidance on analytical approaches to help improve chemical management and policy.

3.19.P-We271 Microplastics Distribution and Occurrence in Greenlandic Fjords

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The northeastern coast of Greenland (Tunu) is hardly, if at all, inhabited and reveals near pristine characteristics. As sea ice still covers the fjords and ocean for most of the year, the population and vessel densities are close to zero. Nevertheless, microplastics (MPs, 1 5,000 µm) and nanoplastics (< 1 µm) are to be found even in places without nearby pollution sources. To date, there is only a limited number of studies on micro- and nanoplastics (MNPs) in the Arctic and numerous questions regarding their transport, distribution, fate, and final consequences for this unique environment remain unanswered.

Within this context microplastic sampling was conducted at eight locations within the fjords of East Greenland and the Denmark Strait with the research vessel Maria S. Merian in August 2022 (cruise MSM110, Ecological tipping cascades in the Arctic seas (ECOTIP)), using the Geesthacht Inert MP Fractionator. Four fractions were taken per sampling station: one hydrocyclone and three filter cartridges with a 300 µm, 100 µm and 10 µm mesh size. 2.5 to 5.2 m³ of ocean water were filtered per sample to process a representative water volume. Transflection-mode infrared spectroscopic measurements were conducted using an Agilent 8700 Laser Direct Infrared (LDIR) Chemical Imaging System.

Measured MP number concentrations ranged between 12 ± 4 L⁻¹ (1 SD, n = 4) at the mouth of the Scoresby Sound and 1.0 ± 0.8 L⁻¹ (1 SD, n = 3) in the Dove Bay. Twelve polymer types were identified in total. PET is by far the most abundant polymer type in all 18 samples with an overall average share of $96 \pm 3\%$ (1 SD, n = 18). Polylactic acid (PLA) is the second most frequent polymer type with an average share of $1.6 \pm 0.5\%$ (1 SD, n = 18).

The fjords of East Greenland and the Denmark Strait are notable hotspots for MP pollution. On average, PET constituted 96% of the total MP abundance, while PLA constituted 1.6%. The level of PLA detected is believed to be the highest ever recorded in marine samples, which raises questions regarding its source and transport. The results revealed no discernible trends between MP concentration, polymer composition, or size distribution and glacial meltwater inputs. Therefore, it is assumed that oceanic currents and sea ice transport exert a substantial influence on the distribution of MPs. The collection of replicate samples proved to be important in demonstrating the temporally varying patchiness of MP distributions.

3.19.P-We272 Global Monitoring of Microplastic Pollution Exceeding 20 Microns: A Study Using Commercial Tanker Ships in the Atlantic, Pacific, and Indian Oceans

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Microplastics (MPs) in the marine environment remain a global concern, as degraded and fragmented MPs pose a heightened risk to marine organisms. While many studies have investigated MPs in oceans worldwide, the diversity in sampling and analytical methodologies has hindered the ability to compare results and fully understand their distribution.

In collaboration with NYK Line, we conducted a global survey of MPs using commercial tanker ships. Seawater was collected from fire hydrants aboard vessels and filtered using 10 µm mesh plankton nets, following a standardized methodology based on Kameda et al.'s approach. This enabled the collection of samples from 198 sites at depths ranging from 5 to 10 meters below the surface. Extracted MPs were

filtered onto PTFE membranes, and micro-FTIR imaging technology combined with identification software YCALOS that we developed was used to accurately identify and quantify MPs larger than 20 µm across the entire membrane surface.

MP concentrations ranged from 23.7 to 4660.0 particles/m³ (n=100), with 15 types of polymers detected, including ABS, alkyd resin, polyacrylate-styrene, epoxy resin, polyamide, PE, PET, PMMA, polyphenylene sulfide, PP, polystyrene, polyurethane, polyvinyl acetate, polyvinyl chloride, and styrene-butadiene rubber.

Using kriging interpolation, contour maps were created to reveal the distribution patterns of each polymer. MPs concentrations were found to be generally higher in the Northern Hemisphere compared to the Southern Hemisphere. In the Atlantic Ocean, a significant outflow of MPs was observed near the Amazon River estuary. Moreover, distinct differences in distribution were noted among various polymer types. These findings provide critical insights into the global distribution and degradation of MPs in the marine environment, enhancing our understanding of marine plastic pollution. The results offer valuable data that can inform strategies for mitigation and foster a more comprehensive understanding of the impacts of MPs on marine ecosystems.

3.19.P-We273 PFAS Presence in Antarctic Drinking Waters

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Per- and polyfluoroalkyl substances (PFAS) are highly persistent substances, known as the forever chemicals. According to scientific results, several deleterious health consequences can be attributed to PFOA and PFOS including hepatotoxicity, growth disturbance, or neurobehavioral, immune and reproductive toxicity. At the moment, drinking water is considered as one of the main exposure routes of PFAS for humans and can be particularly relevant in isolated locations where poor water treatment systems are implemented or long storing periods for drinking water are required.

The aim of the work has been to determine the presence of PFAS in the drinking water consumed at various scientific bases located in the northern Antarctic Peninsula (Vashel Bay, South Orkney Islands, Dundee Island). This is the first study to analyze the presence of PFAS in water for human use in Antarctica.

Briefly, a validated protocol using HLB cartridges was applied to extract PFAS. The analysis was done using high-throughput cutting-edge methodologies based on HRMS (Q Exactive Focus quadrupole-Orbitrap, Thermo-Fisher Scientific) combining ionization sources such as heated electrospray (HESI) and atmospheric pressure chemical ionization (APCI) to sort out the complexity on the analysis of PFAS by extending the analyte coverage of the LC-HRMS methodologies.

Targeted analysis included 42 substances from different families including new emerging PFAS such as ADONA or HFPO-DA (GenX). Preliminary results show that 14 individual PFAS were detected in the drinking water from the bases, including PFBA, PFOA, PFBS, PFOS, FOSA, 6:2 FTCA or 4:2 FOTH. The preliminary results indicate safe levels of PFAS according to EU Drinking Water Directive (2020), according to a limit of 0.5 µg/l for individual regulated PFASs. However, considering the sum of the whole PFAS detected, the established limits are surpassed.

Suspect screening is being conducted using Compound Discoverer 3.3.2.31 (ThermoFisher Scientific) software with a PFAS specifically designed workflow. This analysis aims to detect the max number of fluorinated substances, to uncover the occurrence of other PFAS in drinking water. A system of surveillance and control of the presence of these substances in Antarctic bases should be implemented to protect researchers and itinerant population as well as reduce their environmental presence.

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3.19.P-We274 Cross-Cutting Studies of Per- and Polyfluorinated Alkyl Substances (PFAS) in Arctic Wildlife and Humans

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The Arctic Monitoring and Assessment Program initiated a cross-cutting activity to integrate knowledge across the Arctic region on the presence and impacts of per- and polyfluoroalkyl substances (PFAS). Several PFAS undergo long-range transport via atmospheric (volatile polyfluorinated compounds) and oceanic pathways (perfluorinated alkyl acids, PFAAs), causing widespread contamination of the Arctic. Beyond targeting a few well-known PFAS, applying sum parameters, suspect and non-targeted screening are promising approaches to elucidate predominant sources, transport, and pathways of PFAS in the Arctic environment, wildlife, and humans, and establish their time-trends. Across species, concentrations were dominated by perfluorooctanesulfonic acid (PFOS), followed by perfluorononanoic acid (PFNA); highest concentrations were present in mammalian livers and bird eggs. Time trends were similar for East Greenland ringed seals (*Pusa hispida*) and polar bears (*Ursus maritimus*). In polar bears, PFOS concentrations increased from the 1980s to 2006, with a secondary peak in 2014-2021, while PFNA increased regularly in the Canadian and Greenlandic ringed seals and polar bear livers. Human time trends vary regionally (though lacking for the Russian Arctic), and to the extent local Arctic human populations rely on traditional wildlife diets, such as marine mammals. In accordance to the highest level of PFAS found in East Greenland marine mammals, the human population at the East coast elicited the highest PFAS concentration. Arctic human cohort studies implied that several PFAAs are immunotoxic, carcinogenic or contribute to carcinogenicity, and affect the reproductive, endocrine and cardiometabolic systems. Physiological, endocrine, and reproductive effects linked to PFAS exposure were largely similar among humans, polar bears, and Arctic seabirds. For most polar bear subpopulations across the Arctic, modeled serum concentrations exceeded PFOS levels in human populations, several of which already exceeded the established immunotoxic thresholds for the most severe risk category. Data are typically limited to the western Arctic region and populations. Monitoring of legacy and novel PFAS across the entire Arctic region, combined with proactive community engagement and international restrictions on PFAS production remain critical to mitigate PFAS exposure and its health impacts in the Arctic.

3.19.P-We275 Developing a Risk Assessment Framework for Mineral Based Ocean Alkalinity Enhancement

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Climate change presents an unprecedented challenge, demanding rapid, scalable solutions to address rising greenhouse gas levels and temperatures. Among various approaches, ocean alkalinity enhancement (OAE) has emerged as a promising carbon dioxide removal (CDR) strategy. In addition to boosting the ocean's capacity to absorb and store CO₂, OAE mitigates ocean acidification, offering relief to stressed marine ecosystems. However, scaling OAE requires a robust environmental risk assessment (ERA) framework to evaluate potential impacts, such as those of alkalinity feedstocks on marine life. This framework should integrate lab studies, field experiments, and modeling to adapt to diverse implementation scenarios. Hourglass is developing a predictive tool for evaluating the ecotoxicological impacts of OAE to support responsible project design, permitting, and scaling of this CDR solution for climate mitigation. This ERA focuses on the dissolution products of mineral alkalinity feedstocks like olivine, particularly the bioavailability and toxicity of nickel the primary trace metal found in olivine. By combining fate and transport modeling, biotic ligand modeling (BLM), and toxicokinetic-toxicodynamic (TKTD) modeling, the ERA simulates nickel exposure scenarios based on olivine application data, predicting effects on marine species and populations.

In particular, Hourglass is using The General Unified Threshold model of Survival (GUTS) to simulate nickel exposure scenarios based on field data, contributing to the development of a comprehensive ERA framework. GUTS, recognized by the European Food Safety Authority in 2018 for assessing pesticide risks to aquatic organisms, is a natural choice for TKTD modeling in olivine applications. By simulating

survival across varied exposure scenarios, GUTS helps establish safe concentration thresholds and exposure durations. Its open-source, user-friendly design will help support wide-spread adoption by the emerging CDR industry.

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3.19.P-We276 Climate Change and Permafrost Thaw Impact on Drinking Water Quality in Greenland

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Permafrost underlies 22% of the Northern Hemisphere's exposed land surface and is thawing at an alarming rate as a direct consequence of climate change. Permafrost thaw may potentially release stored organic matter and contaminants into the environment. Contaminants, including heavy metals, persistent organic pollutants and infectious agents locked in permafrost, may present a risk for both human and animal health. This project aims to understand the impact of permafrost thaw to water quality and thus health in Greenland. To evaluate the impact on drinking water quality in Greenland different sites spanning the west coast have been selected. Since most of the Greenlandic communities are supplied with water sourced from lakes, the sampling focus is evolving around this. To get a first evaluation of the recent historic pollution in water lakes in Greenland, 50 cm sediment cores have been collected from the drinking water lakes in Sisimiut (in the sporadic permafrost zone) and Ilulissat (in the continuous permafrost zone), and divided into 4.5 cm increments, which will be dated via the ²¹⁰Pb dating method. The dated cores will be analyzed for their content of nutrients (phosphorous and nitrogen) and organic matter, organic local and long distance transported contaminants (polychlorinated biphenyls and fuel products including polycyclic aromatic hydrocarbons), local organic contaminants (fuel products), heavy metals and radionuclides. Alongside the chemical assessment, presence of cyanobacteria and fecal contaminants will also be evaluated by dPCR analysis on extracted DNA. The results will provide a solid offset for our further investigations to create an understanding of how historic and current contaminant emissions combined with climate change related temperature increases affect the quality of freshwaters in an area with permanently frozen ground.

3.19.P-We277 Pollution of Microplastics in the Pearl River Estuary of China: From the Perspective of Multiple Environmental Media

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The estuarine hydrological changes lead to the dramatic changes of pollutants in estuary. A comprehensive understanding of the changing characteristics of estuarine pollutants is essential for environmental management. This study investigated the abundance and concentration, seasonal variations of microplastics in groundwater, water column, and sediments in the Pearl River estuary using FITR and py-GC/MS. The results found that the average abundance of microplastics in groundwater was 2694 p/m³ with an average concentration of 150.47 mg/m³. In the water column, the average abundance was 243.86 p/m³ with an average concentration of 0.52 mg/m³ in winter, and 112.27 p/m³ with an average concentration of 0.21 mg/m³ in summer. In sediment, the average abundance was 117.78 n/kg with an average concentration of 4.24 mg/kg. Microplastics exhibited spatial and temporal variability in their pollution characteristics across different environmental media. The level of PVC contamination in the water column was underestimated, and a large amount of PP and PE was deposited in the sediments. FITR-corrected py-GC/MS was used to determine the loadings and contributions of microplastics. The Pearl River estuary transported a total of 1605.3 t of microplastics during the dry season, Surface runoff contributed the largest amount, accounting for 67.52% of the total. Besides, it was important to consider the contribution of groundwater and sediments. This work provided a comprehensive analysis of the environmental fate of microplastics in various environmental media, quantified their concentration, and enhanced our understanding of microplastic fluxes to the sea in estuaries.

3.19.P-We278 Unraveling the Effects of Microfibers Across Treatment stages via a critical prey species, in the context of climate change

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Mysid shrimp are an important prey item in marine and freshwater food webs globally, including those

containing key predators such as gray whales and salmonids. Gray whales require millions of zooplankton daily, primarily mysids, and are threatened by worsening climate conditions such as ocean acidification. Additionally, whales can consume over 20 million microparticles (primarily microfibers) daily from mysid prey. Microfibers exert chemical and physical toxicity, but knowledge is sparse regarding how much each contributes to organismal stress. Therefore, our goals were to further estimate impacts to coastal food webs and to better understand the contribution of chemical vs. physical effects from microfibers, in addition to understanding impacts in the context of climate change on these critical prey species. We quantified responses to polyester microfibers across treatment stages (grunge/naïve, ozonated, chemically modified) and weathering states, including low pH exposure, on native and surrogate mysids. Briefly, microfibers were produced from textiles across treatment stages provided by an anonymous clothing manufacturer. Mysids were exposed to 5 (*A. bahia*, purchased) or 10 microfibers (*H. sculpta*, native field collected) / mL for 7 days or 48 hours, respectively. Following exposures, *A. bahia* underwent a sublethal low pH challenge. Behavioral toxicity was measured in both species. Results demonstrate that growth was reduced in *A. bahia*, and behavioural responses are predominately hypoactive, being most sensitive to chemical treatment or weathering, and potentially exacerbated at a lower pH. Behavioural impacts are more pronounced in native *H. sculpta*. These effects have broad implications for individual, population and ecosystem health. Current studies investigate the same fabric treatments in cotton in both species, including RNA seq analysis, and seek to better understand microfiber occurrence in relevant coastal areas. To our knowledge, this is one of the first studies utilizing textile microfibers across known treatment stages in both native and surrogate (model) species and could directly inform more sustainable textile production in the future.

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3.19.P-We279 Per- and Polyfluoroalkyl Substances (PFAS) in Sub-Antarctic Seabirds: Evidence for Long-Range Transport and Bioaccumulation of Emerging Contaminants

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Poly- and perfluoroalkyl substances (PFAS) are a large group of chemicals that are characterised by a hydrophilic moiety (e.g., SO₃⁻ or COO⁻) and a hydrophobic perfluorocarbon chain that varies in length. PFAS display unique properties due to combined oil and water repellence, extreme stability, and hence are used in a wide range of industrial processes and consumer products. These chemicals can be problematic in the environment due to their potential to undergo long-range transport, persistence and toxicity. Some longer-chain PFAS have already been listed in the Annexes of the Stockholm Convention and included in other international regulations, but replacements have started to emerge. Furthermore, the European Chemicals Agency (ECHA) is seeking to ban the use and production of PFAS (defined as chemicals containing CF₂) completely in Europe. As such, it is important to document current levels of both legacy and emerging PFAS in the environment prior to this proposed ban so that any resulting decline in concentrations can be assessed accurately. Here, we employ an ultra-performance liquid chromatography coupled to a high-resolution mass spectrometer (Orbitrap) to analyse a range of both legacy and emerging PFAS including 11 PFCAs (C₄-C₁₄), 9 PFSAAs (C₄-C₁₀), 3 FASAs, 3 PFPiAs and 6 fluorotelomer acids, among others. We have analysed the liver tissues of 78 seabirds, from 13 different species sampled from South Georgia between 2018 and 2022. The data show varying spatial and temporal trends between different species and we draw comparisons between trophic levels by employing stable isotope analysis. Trends in these data reveal various pathways e.g., atmospheric and hydrospheric, for PFAS into the Southern Ocean before bioaccumulating through food chains. Our research addresses the need to understand exposure levels in wildlife to PFAS compounds in areas close to and remote from sources.

3.19.P-We280 Occurrence of Chemicals of Emerging Concern in Antarctic Soil

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Chemicals of emerging concern (CECs), including pharmaceuticals, personal care products, and industrial chemicals, are increasingly detected in remote environments due to anthropogenic activities. This study investigates the occurrence and distribution of CECs, e.g. phthalate esters, organophosphate esters, UV filters and musk fragrances in Antarctic soils. Soil samples were collected near the Chinese Zhongshan Station, located on the Larsemann Hills of east Antarctica. This region experiences a warming trend, is relatively humid, and has a sub-Antarctic maritime climate. The vegetation in this area is very sparse. Samples were collected in 2008, 2014, and 2019 from five locations, including below-peak soil, intertidal soil, and sediment. After Soxhlet extraction, samples were analyzed using an Agilent gas chromatograph coupled to a triple quadrupole mass spectrometer (LC-MS/MS). Results revealed the presence of a range of contaminants, including synthetic musk, plasticizers and flame retardants, with concentrations varying from different locations. These findings indicate both long-range transport and localized sources of pollution. The persistence of these chemicals raises concerns about their potential ecological impacts on Antarctic flora and fauna, which are adapted to the Antarctic environment. This study underscores the need for improved waste management practices at research stations and highlights the importance of monitoring CECs in fragile ecosystems to mitigate their long-term environmental consequences.

3.19.P-We281 Local Sources Versus Long-Range Transport of POPs and CEACs in the Arctic: Future Developments Related to Climate Change

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Local sources of persistent organic pollutants (POPs) and Chemicals of Emerging Arctic Concern (CEACs) from use in communities, shipping, and industrial activity contribute to Arctic contamination as does long-range environmental transport. Increased human activity in the Arctic as the climate warms may enhance the significance of local sources. Furthermore, climate change may lead to secondary sources from existing reservoirs of POPs and CEACs in the Arctic. This review examines the emerging evidence for releases from these secondary sources of formerly deposited POPs and CEACs as the Arctic climate warms and the potential for future releases with increased infrastructure development and economic activity. Arctic permafrost degradation represents an important source of natural and anthropogenic polycyclic aromatic hydrocarbons (PAHs), and indications of POP releases related to permafrost thaw, from previous deposition as well as waste sites. Deposition of POPs and some CEACs to Arctic glaciers is relatively well studied while fewer studies explore the impacts of remobilization. Expansion of economic development may increase emissions or create new sources of CEACs in the Arctic. The predicted northward expansion of agriculture, aquaculture, and ship traffic could bring increased emissions of CEACs to northern waters, including pesticides not previously used in the Arctic. Increased industrial chemical use, such as fire-fighting foams, flame retardants, and lubricant and plastic additives, is likely to occur following the expansion of infrastructure such as airports, seaports, mining, and oil and gas development. While PAHs are relatively well-studied, there is an urgent need for environmental measurements and modelling of emissions of CEACs associated with the expansion of economic activity in the Arctic as well as to predict the future release of legacy POPs from secondary sources, particularly from permafrost.

3.19.P-We282 Identifying Bioaccumulative Emerging Per- and Polyfluoroalkyl Substances in a Coastal Food Web

Yuefei Ruan¹, Qi Wang¹, Kenneth M.Y. Leung¹ and Paul K.S. Lam², (1)City University of Hong Kong, Hong Kong (Greater China), (2)Hong Kong Metropolitan University, Hong Kong (Greater China) The trophodynamics of many emerging per- and polyfluoroalkyl substances (PFASs) in aquatic food webs remain poorly understood. Here, coastal seawater and marine organism samples were collected from the northern South China Sea to investigate the trophic biomagnification potential of legacy and emerging PFASs. Significant trophic magnification was observed for 22 PFASs, where the trophic magnification

factors of cis- and trans-perfluoroethylcyclohexane sulfonate (PFECHS) isomers were reported for the first time. Perfluorohexanoic acid was trophic-magnified, possibly attributed to the PFAS precursor degradation.

Our study is extended to lab-based validation. The toxicokinetics of emerging PFASs have rarely been reported. Here, tissue-specific uptake and depuration kinetics of PFECHS and 6:2 and 8:2 chlorinated polyfluoroalkyl ether sulfonates were studied in marine medaka (*Oryzias melastigma*). Evident bioconcentration was found for these three PFASs in the exposed fish, which showed longer residence times in eyes than in other tissues; trans-PFECHS showed higher bioconcentration potential than cis-PFECHS, and PFECHS exposure resulted in significant alterations in multiple proteins associated with eye function in medaka.

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3.20 Chemical Fate in the Soil-Plant-System and Related Impacts and Risks

3.20.T-01 Uptake of Tire-Derived Compounds by Lettuce Grown in Soils

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Tire wear particles and associated organic compounds (tire-derived compounds, TDCs) enter agricultural environments via irrigation with treated wastewater and application of biosolids. In recently published work, multiple TDCs were detected in commercial leafy vegetables, at concentrations up to 665 ng/g dry weight, confirming that TDCs are present in the agricultural environment and accumulate in edible plants. Lettuce uptake and translocation of five TDCs, namely benzothiazole, 1,3-diphenylguanidine (DPG), hexamethoxymethylmelamine (HMMM), N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD), and 6PPD-quinone was previously studied hydroponically. In hydroponic experiments, compounds are assumed to be completely bioaccessible for plant uptake, whereas in soil systems mainly the fraction of compounds dissolved in soil pore water is bioaccessible. In this study, we investigated the uptake of the same TDCs by lettuce plants grown in three soils, where TDCs were continuously introduced to the soil-plant system via spiked irrigation water over twenty-six days. TDCs were detected in lettuce grown in all three soil types (1.1–94.4 ng/g), but at very different concentrations than in hydroponic studies. These differences could be attributed to the bioaccessibility of TDCs in soils. Sampling of soil at different depths revealed that vertical mobility was low, and that all TDCs were predominantly retained in the root zones of plants. However, bioaccessibility was reduced by the formation of non-extractable residues in soils, especially for HMMM and 6PPD, which reduced their accumulation in lettuce plants. Bioconcentration factors, calculated as the ratio of TDCs measured in lettuce leaves to the extractable fraction of TDCs in soil, were in agreement with results from our hydroponic study, confirming that lettuce uptake and translocation processes were consistent between the two systems. Accumulation of all studied TDCs (including both neutral and cationic species) was greatest in lettuce grown in soil with a high sand and low clay content, and lowest in lettuce grown in soil with a low sand and high clay content. Together with previously published work, these novel results demonstrate that both plant uptake and translocation, as well as bioaccessibility in soil determine the accumulation of tire-derived compounds in leafy vegetables, which introduces these contaminants of emerging concern into the food supply.

3.20.T-02 Do Persistent and Mobile Chemicals Impact Agricultural Wastewater Reuse? – Accumulation in Arugula

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With increasing water stress agricultural wastewater reuse is becoming increasingly relevant worldwide,

which leads to higher demands on wastewater quality to minimize detrimental effects of human and environmental exposure with contaminants. Persistent and mobile (PM) chemicals are difficult to remove from wastewater, many of them passing even advanced wastewater treatment without significant concentration reduction. Consequently, these PM chemicals seem highly relevant for wastewater reuse, yet little is known on their up-take by plants. This study investigates the uptake by and distribution in arugula (*Eruca sativa* var. Speedy) for 65 PM chemicals in pot experiments with spiked water. Water uptake of the plants was controlled to calculate the maximum theoretical uptake (TMU; amount of irrigation water taken up * concentration in irrigation water). Uptake into edible parts of arugula exceeded 10% of the TMU for 18 PM chemicals and reached up to 64% of the TMU for perfluoropropionic acid. The median plant uptake into edible parts was 2.8 times higher for PM chemicals studied herein compared to 50 less polar anthropogenic chemicals from literature ($p = 0.05$) while the median translocation factor describing the distribution between roots and the edible parts of the plant was six times higher for the PM chemicals tested compared to 98 chemicals from literature studies ($p = 0.005$). These results are in contrast to established models for plant uptake often predicting a reduced uptake for very polar chemicals. In irrigation experiments with reclaimed wastewater 25 PM chemicals were detected in arugula leaves with concentrations of up to 115 ng/g fresh weight. A risk assessment based on the toxicological threshold of concern showed that no critical exposure can be reached with normal consumption levels. However, for more than half of the PM chemicals with a TMU > 10% experimental toxicity data is missing and additional exposure pathways (e.g. drinking water) may contribute significantly to the total human exposure with PM chemicals. Consequently, PM chemicals should be evaluated critically in the context of agricultural wastewater reuse as they seem to combine a low potential for removal during water treatment with an increased potential for plant uptake.

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3.20.T-03 Passive Sampling in Soil

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The viability of using passive sampling for plant protection products in soils is presented. Currently available passive sampling technologies are lacking due to high costs, poor scalability and difficulty of use. A 3D-printed passive sampling device (3D-PSD), previously validated for water analysis, was repurposed for soil deployment. An analytical method was first developed, and its performance was evaluated for the quantitative determination of 18 compounds in passive sampler extracts, soil and leachate with respect to linearity, range, accuracy, precision, limits of detection and limits quantitation. The 3D-PSD was able to capture a total of two-thirds of all compounds spiked in a controlled laboratory test system ranging from compounds more polar in nature, such as sulfamethoxazole, which was not detected in soil but was found in the leachate during simulated rainfall events. To less polar compounds such as Pyraclostrobin were detected both in soil and on disk. Carbamazepine and its transformation product, Carbamazepine-10,11 epoxide, were detected in high concentrations in the leachate, soil and disk samples, due to their presence already being detected in the soil substrate. In the field tests a total of six spiking events were studied. The 3D-PSD detected half of the spiked compounds and one transformation product of an originally spiked parent compound, CGA321113 a Trifloxystrobin derivative. The 3D-PSDs were field tested in garden soil for 21 days. Pet parasiticides such as Imidacloprid and turf antifungals like propiconazole were detected on disk using liquid chromatography-high resolution mass spectrometry and compared against a commercial library of 1219 compounds. This work is so far the first to demonstrate the application of passive sampling for plant protection products to understand their stability and movement in the soil environment.

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3.20.T-04 The Enantiomeric Uptake, Translocation, and Metabolism of Ibuprofen in Rice (*Oryza sativa* L.)

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The enantiomeric uptake, translocation, and metabolism of ibuprofen (IBP), a commonly used pharmaceutical and personal care product (PPCP), in rice have not been well understood. In this study, we investigated the enantiomer-specific patterns and mechanisms of IBP uptake, translocation, and metabolism using a hydroponic rice model. Our results showed that rice tissues exhibit a stronger uptake capacity for the R-enantiomer (R-IBP) compared to the S-enantiomer (S-IBP), with R-IBP also demonstrating higher translocation from roots to shoots. We identified seven metabolites in rice tissues, suggesting enantiomer-specific metabolic pathways. Transcriptomic analysis revealed that differentially expressed genes (DEGs) were significantly enriched in pathways related to Photosynthesis, Ribosome, Nitrogen metabolism, ABC transporters, MAPK signaling, and Photosynthesis (Kyoto Encyclopedia of Genes and Genomes, KEGG). Molecular docking analysis further indicated that R-IBP exhibited stronger binding affinity to key metabolic enzymes compared to S-IBP, supporting the observed differences in metabolism. This study provides novel insights into the enantiomeric behaviors of ibuprofen in crops, contributing to a deeper understanding of PPCP fate and effects in agricultural systems.

3.20.T-05 Terrestrial Pollution: A Case Study of Fate and Impact of Antibiotics on Soil Microbes **Oluyemi Ojo**, *Environment, University of York, York, United Kingdom*

Pharmaceuticals and personal care products (PPCPs) are perceived as contaminants of emerging concerns due to their inherent ability to induce physiological changes at low doses in non-target species in the environment. Despite the increased use of PPCPs, their environmental ecotoxicological profile is yet to be established. This study is focused on bridging this gap in the terrestrial ecosystem. The overall aim of this research is to investigate the effects of pharmaceuticals and personal care products on terrestrial habitat. A systematic review of previous publications and ecotoxicological databases revealed PPCPs have an inhibitory or lethal effect on the biochemistry, physiology, population, and yield of some plants, soil microbiota, and soil invertebrates. A total of 2678 publications were identified exploring the antimicrobial fate, occurrence, and effects in the soil environment. A total of 857 soil entries were found in the Umweltbundesamt database (UBA 2021). The most highly monitored antimicrobials in soils were tetracycline, oxytetracycline, and chlortetracycline, and the antimicrobials detected at the highest concentrations were enrofloxacin, ciprofloxacin, and chlortetracycline. The highest measured environmental concentration of antimicrobials was found in South America, Asia, and Europe. Effects data were found for 11 of the top 15 antimicrobials monitored. Of these, only 2 had data on all key ecological components of the soil environment (microbes, invertebrates, and plants). Based on the ubiquity of these PPCPs, a risk assessment was carried out using the risk quotient (RQ) approach. Risk characterization of these chemicals indicates that sulfonamides, tetracyclines, and fluoroquinolones are of environmental concern as they have risk quotients greater than 1, i.e., $RQ > 1$. Furthermore, several knowledge gaps have been identified. This includes toxicity data for chemicals and the degree to which the ecotoxicological effects of PPCPs could disrupt ecosystem service delivery is yet to be well elucidated. The impacts of PPCPs on vital microbial functions at the soil-rhizosphere are also yet to be constrained. Persistence, effects, and adsorption studies were carried out on five target APIs. The results generated have shown the probable toxicological effects of these antibiotics on terrestrial microbes. The results from this study would inform policies on the management of potential threats from PPCPs.

3.20.P Chemical Fate in the Soil-Plant-System and Related Impacts and Risks

3.20.P-We283 Forest Plant Uptake of Metals Inherited from the Great War

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The highly localised fighting of World War I (WWI) resulted in large amounts of munitions concentrated along the former front line in northern and eastern France. These munitions represent a toxic legacy that is still buried in the ground. Their degradation can now be considered as a source of trace metal contamination throughout this area, and the absorption of these metals by forest plants may induce changes in plant functional traits.

Our research aims at determining the fate of metals in soils and plants on former battlefields, across different environments and in presence of other potential contaminants such as explosives. For this purpose, we surveyed two regions with contrasted vegetation and soil properties (substrate, topography, soil pH and organic matter content). Samples from each matrix (soil, water, plant tissue) were taken into

woodlands affected by the fighting and from unaffected woodlands (controls), and subsequently analysed for a range of trace metal elements.

Higher concentrations in soils and waters were measured for some metals in the red zone (i.e. the area completely destroyed by the conflict) compared to the control zone. Our results enable to validate the hypothesis of a WWI-associated soil contamination by trace metals and highlight their mobility. The analyses of metal contamination in plant tissues allow to compare their uptake from the soils located in the red zone compared to the control zone. These results will contribute to identify the metals with the highest environmental impact, and to relate them to alteration in plants life-history traits measured in our study.

3.20.P-We284 Interactions Between Reclaimed Water Irrigation and Planting Season Revealed by Non-Target HRMS Rice Fingerprinting

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Reclaimed water irrigation is a potential resolution to mitigate agricultural water scarcity, especially for rice irrigation. However, the effects of reclaimed water irrigation on rice are not clarified, including its interaction with seasonal variations. Aiming to elucidate the relationship between reclaimed water irrigation, planting season, and the potential physiological effects on rice, this study performed non-target small-molecule fingerprinting in rice using high-resolution mass spectrometry (HRMS).

Rice plants were cultivated in August-December 2023 and March-July 2024. Rice plants were sampled for each irrigation group (reclaimed or river water, each $n = 35$) at the end of each season. Homogenized white rice samples were processed through solvent extraction and cartridge cleanup. The samples were analyzed using a liquid chromatography orbitrap mass spectrometry system. The molecular features with high variabilities (relative standard deviations $> 30\%$) were filtered. The distribution of rice samples was illustrated using orthogonal partial least squares discriminant analysis (OPLS-DA) models. Features with scores of variable importance in projection greater than one, significant differences between groups ($p < 0.01$), and fold changes of relative concentrations greater than two were selected as candidate markers. The OPLS-DA models that were constructed using 2152 features discriminated between two planting seasons and between two irrigation waters ($R^2Y > 0.643$, $Q^2 > 0.573$). Seventy-one molecules were selected and identified as markers of planting season. They were mainly endogenous compounds and more abundant in rice planted in 2024 than in 2023. For rice grown in 2023, 102 molecules were selected and identified as markers of irrigation water, while only 26 molecules were for 2024. These markers were primarily endogenous compounds. In the rice grown in 2023, the markers were more abundant in the rice irrigated with reclaimed water than those with river water. However, the rice grown in 2024 revealed an opposite trend.

HRMS non-target analysis was successfully applied to illustrating the small-molecule distributions in rice. The results demonstrated that the small-molecule fingerprints in rice differed by the irrigation water used, which interacted with planting season. Further findings on the effects on rice may provide crop rotation suggestions to improve the implementation of reclaimed water irrigation.

3.20.P-We285 Reactive Transport of Coexisting Organic Pollutants and Nitrate

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Background: Soil and groundwater contamination is globally widespread. Soil microbes may biodegrade organic contaminants by oxidising them, which requires electron acceptors. In agricultural soils, organic contaminants typically coexist with nitrate, a soil contaminant and electron acceptor. Soils are not well-mixed reactors. Hence, even if organic contaminants, electron acceptors, and microbes are present, their spatial distributions might not overlap, and biodegradation may not occur. To address this, we studied how soil hydrology affects overall contaminant mixing, transport, and biodegradation. We compared models of various complexities to simulate contaminant transport, mixing, and fate for cases where veterinary pharmaceuticals (VP) and nitrate from manure coexist, to identify what models should be prioritized for such research.

Methods: We first modeled 1D soil columns to estimate VP leaching from manured soils, without considering mixing with nitrate. 1D models are fast to compute, and can be applied spatially distributed to obtain broad overviews at regional scales. We then developed leaching models with more complexity (2D space, microbe-growth- and mixing-dependent biodegradation rates, soil heterogeneity, transient fluxes,

etc). However, we omitted contaminant adsorption to limit the overall model complexity. Nevertheless, we recognize the importance of sorption, and sorption interactions on VP transport, and also conducted sorption batch experiments of multiple VPs in manure in soils, to study mixture and nitrate related effects on VP adsorption.

Results: The 1D results underscore the need to consider a combination of factors (e.g., mixing and reaction rates) in assessing VP leaching, which varies by compound. Even with the additional complexities of the 2D model, biodegradation outcomes for various contaminants with various biodegradation parameters are ranked similarly as in the 1D model, except perturbed with random noise. The sorption experiments showed that nitrate influenced VP sorption. Thus, if VP and nitrate coexist in soils as mixtures, which generally occurs under organic manure, then additive, synergistic, or antagonistic interactions on sorption must be expected.

Conclusion: It may be most efficient to use 1D models, but account for additional complexity as a statistical uncertainty by using a range of parameter values. In future studies, we will integrate insights from the VP sorption experiments into reactive transport models.

3.20.P-We286 Composted Biosolids and Biochar as Sources of Wastewater Derived Contaminants for Plant Uptake

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Climate change, overuse of water resources, and population growth have led to the depletion of freshwater sources. To overcome these challenges, treated wastewater was introduced into the water cycle. However, studies have shown that wastewater treatment plants do not completely remove organic pollutants, such as pharmaceuticals, corrosion inhibitors, PFAS, etc. Therefore, the use of treated wastewater for irrigation or composted sewage sludge (i.e., composted biosolids) as fertilizer exposes the agricultural environment to wastewater-derived contaminants, which may accumulate in plants. Thus, the main objective of this study is to explore cost-effective methods to reduce plant uptake of these contaminants by applying composted biosolids and/or biochar (pyrolyzed biosolids) to the soil. We hypothesized that these amendments would increase the number of sorption sites and enhance the soil's affinity for wastewater-derived organic contaminants, thereby reducing their bioavailability through sorption processes.

The experiment examined the uptake of organic contaminants in alfalfa plants grown over 180 days, with six harvests. The plants were grown in soils mixed with composted biosolids or biochar (or their combination) and irrigated with treated wastewater or fresh water. Our results indicate that while composted biosolids could serve both as a source of contaminants when combined with freshwater irrigation, and as a sink (i.e., sorbent) when combined with treated wastewater irrigation, biochar primarily served as a significant sorbent due to its high surface area and hydrophobic properties. For example, the average concentration of carbamazepine in alfalfa leaves was highest in plants grown in soils irrigated with treated wastewater without application of organic amendment (mean concentration of 15 ng/g). In contrast, plants grown in soils amended with composted biosolids or biochar exhibited a 25% and 80% reduction in carbamazepine concentration in the leaves, respectively. These results were consistent across all five harvests. Our data demonstrate that application of composted biosolids or biochar significantly reduces the plant uptake of organic contaminants, probably due to a decrease in their bioavailability due to sorption processes, thereby reducing human exposure to contaminants of emerging concern.

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3.20.P-We287 Land Application of Biosolids: Bioaccumulation of Emerging Contaminants in Vegetables

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Biosolids, the nutrient-rich organic materials resulting from the treatment of sewage sludge, can be land-applied to improve plant health and soil quality in addition to offering benefits over detrimental disposal methods such as landfilling and incineration. However, biosolids contain numerous unregulated organic chemicals (UOCs) leading to concerns that may impede public perception of the benefits gained from land

application, potentially hindering the expansion of its beneficial use. Therefore, understanding the broad human and environmental health risks of biosolids-borne UOCs is imperative for the safe use of biosolids in agriculture. In this study, biosolids were land-applied to vegetable crops (sweet corn, cucumber, potato) at agronomically realistic rates for one growing season. Thin-film passive samplers were deployed in the field plots as a biomimetic tool to estimate the bioavailability of biosolids-borne UOCs. Passive samplers, soil samples, and the edible tissues of various vegetables were collected and analyzed for 20 priority UOCs. The concentrations quantified in the samples evaluate the bioavailability, uptake, and presence of the priority UOCs in food crops. In future work, biosolids will be applied to fruit trees at agronomically realistic rates to determine the bioaccumulation of UOCs in the edible portion of the fruits. These completed studies will contribute to holistic human health and ecological risk assessments.

3.20.P-We288 Assessment of the Bioaccumulation, Translocation, and Distribution of Per- and Polyfluoroalkyl Substances in Terrestrial Plants

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Per- and polyfluoroalkyl substances (PFAS) are synthetic pollutants with persistent and bioaccumulative properties, raising environmental and human health concerns. Terrestrial plants can absorb PFAS through their roots from soil in contaminated areas. However, research on species-specific PFAS accumulation and distribution in plants, especially regarding short-chain emerging PFAS and PFAS mixtures, remains limited.

This research focused on four plant species common sunflower (*Helianthus annuus* L.), barley (*Hordeum vulgare* L.), broad bean (*Vicia faba* L.), and spearmint (*Mentha spicata* L.) to unravel the bioaccumulation, translocation, and distribution patterns of nine environmentally relevant PFAS. In greenhouse experiments, individual plants were exposed to soil spiked with varying concentrations of a PFAS mixture. After 60 days of exposure, plant parts, including roots, leaves, stems, and apices, were harvested and their PFAS content was determined.

During the experiment, aphids infected several plants, especially broad beans exposed to the highest PFAS concentrations, which showed upwardly folded leaves with yellow/brown tips symptoms typical of Bean leaf roll virus (BLRV). An enzyme-linked immunosorbent assay (ELISA) could not confirm the presence of BLRV. Although our study provides initial observations, further research is needed to determine whether PFAS exposure increases plant susceptibility to aphid infestation and to clarify if the observed abnormal phenotypic effects are directly caused by PFAS or if PFAS exposure amplifies the plant's response to aphids or other stressors. These aspects, however, are beyond the scope of the current study. In further analyses, scheduled for early 2025, the following endpoints will be determined for each species: i) the total PFAS loading of the aboveground biomass and the PFAS loading within each aboveground tissue; ii) the relative and absolute PFAS concentrations of the aboveground biomass and the PFAS concentration of each aboveground tissue separately; iii) the bioaccumulation factors for the aboveground biomass and the bioaccumulation factor of each aboveground tissue separately; and iv) the translocation factors between the roots and the aboveground tissues. We expect our findings to advance the application of phytoremediation for PFAS-contaminated soils and to provide key insights into specific PFAS accumulation sites within plants, which is essential for evaluating potential consumption risks.

3.20.P-We289 Imidacloprid Uptake and Accumulation in Lettuce Plant (*Lactuca sativa* L. var. *longipolia*) and Its Effects on Abundance of Microbial Communities in Cultivated and Non-Cultivated Arid Soil

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Systemic plant protection products, such as neonicotinoids (NIs), are capable of being translocated throughout a plant. Although NIs are less toxic to mammals, fish, and birds, their impact on microbial and non-target insects is of concern. This study investigates the uptake, translocation, and accumulation of the NI, imidacloprid (IMI), in romaine lettuce (*Lactuca sativa* L. var. *longipolia*). Exposing 15-day-old seedlings to 10 mg/L of IMI, the effects on microbial communities in both cultivated (CS) and non-cultivated soil (NCS) were studied along with IMI translocation within plant tissues. The concentrations of IMI in soil varied temporally and between soil types after initial application, with a decrease from 2.0 and 7.7 mg/kg on the first day of sampling to 0.5

and 2.6 mg/kg on the final sampling day (day 35) for CS and NCS, respectively. The half-life of IMI soil was 10.7 and 72.5 days in CS and NCS, respectively, indicating that IMI degraded more quickly in CS, possibly due to smaller grain size, aeration, microbial degradation, and water flow. The accumulated concentrations of IMI in lettuce tissues ranged from 12.4 ± 0.2 and 18.7 ± 0.9 mg/kg in CS and NCS, respectively. The highest concentration of IMI was found in the shoots, followed by the roots, whereas the soil showed the lowest IMI residuals at the end of the trial. Soil bacteria and fungi were altered by the application of IMI, with a lower abundance index within the bacterial community, indicating a negative impact on the distribution of bacteria in the soil. The results of this study have important implications, especially for understanding the dynamics of NI uptake and distribution in lettuce in cultivated and non-cultivated Arid Soil. Understanding how these chemicals move within plants, such as lettuce, provides valuable insights that can be applied to agricultural practices, food safety assessments, and regulatory decision-making concerning NIs.

3.20.P-We290 Absorption and Translocation of 14C-Glyphosate in Neotropical Weed *Machaerium hirtum* (Vell.) Stellfeld

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The use of radioactivity in agriculture allows for a range of insights, particularly in understanding the effects of herbicides on weeds. In this context, the present study aimed to determine the absorption and translocation of carbon-14-labeled glyphosate (14C-glyphosate) in neotropical weed *Machaerium hirtum* (Vell.) Stellfeld (Leguminosae-Papilionoideae-Dalbergiaceae). The study was carried out in a completely randomized design, and the treatments were evaluated 3, 48, and 72 hours after application (HAA), with three replications. The plants had four fully developed true leaves at the time of the 14C-glyphosate application, which was performed on the adaxial surface of the leaf (3,299.82 Bq). Quantitative evaluation was performed via liquid scintillation spectrometry (LSS) by 5 min, on the segments root, stem, treated leaf, leaves below the treated leaf, and leaves above the treated leaf, after combustion in the biological oxidizer by 3 min. Semi-quantitative evaluation was done using autoradiography of each experimental unit with a radioscaner. The data obtained were transformed into percentages of radioactivity in each weed segment to determine absorption and translocation. At 3 HAA, radioactivity was detected in all parts of the weed, with 77.01% of the absorbed total retained in the treated leaf. At 48 HAA, although 94.15% of the applied radioactivity was not absorbed, there was an increase in absorption and translocation of the herbicide, with 5.54, 0.13, 0.08, 0.04, and 0.06% of the radioactivity present in the treated leaf, root, leaves below, leaves above, and stem, respectively. At 72 HAA, greater translocation was observed in weed, reaching 0.37% for the other parts of the plant (except the treated leaf). It was concluded that 14C-glyphosate exhibits a low percentage of absorption and translocation in *M. hirtum* throughout the evaluated period, which can be attributed to the morphological and anatomical characteristics of the neotropical weed.

3.20.P-We291 Roots of Concern: Addressing PFAS Contamination in Vegetation at a Former Military Airbase

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Measuring per- and polyfluoroalkyl substances (PFAS) in vegetation is crucial for understanding the environmental implications of these persistent contaminants. While PFAS uptake by plants presents opportunities for innovative remediation strategies such as phytoremediation, it also poses potential human and ecological risks and challenges during site redevelopment. Activities such as tree removal, mulching, or composting may reintroduce PFAS into the soil, complicating remediation efforts and raising concerns about ecological integrity and human exposure.

Our study focuses on a former military airbase, where an extensive PFAS contamination in soil (contaminated area > 11.000 m²) and groundwater (> 3 million m³) is linked to the historical use of Aqueous Film-Forming Foam (AFFF) in firefighting operations from ~1960 to 2006. The site features a sandy soil and a groundwater depth of 8 to 10 meters, beyond the reach of tree roots. An inventory of PFAS in current onsite vegetation (trees and grass) was conducted at locations with elevated soil concentration, as soil remediation and redevelopment into a residential area is planned. We measured PFAS in four tree species analyzing soil, litter, trunks, and leaves. Most sampled trees were situated in soils with PFAS concentrations exceeding Dutch threshold levels (> 59 µg/kg dry weight) in the upper 2 meters. We also assessed PFAS levels in three grass plots located at contaminated soils. Elevated PFAS concentrations were observed in vegetation corresponding to high soil levels, with leaf concentrations

generally higher than those in woody material. Among the studied species, beech trees had the highest PFAS concentrations, while birch trees had the lowest. Short-chain PFAS were more prevalent in vegetation than in soil, likely due to their greater water solubility. The presence of PFAS in vegetation complicates future management of the former military airbase. Leaf litter from contaminated trees can lead to soil recontamination after soil remediation. Current disposal options for organic material with PFAS are limited, raising long-term management concerns. Additionally, grass from heavily contaminated areas may further contribute to soil contamination if not managed properly. To prevent migration of PFAS, mowed grass and other biomass from these heavily contaminated areas should be separated from other non/less contaminated areas and needs to be treated/processed properly in order to remove/contain PFAS.

3.20.P-We292 The Impact of Container Size on the Plant Uptake of Pharmaceuticals from Soil

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Soil can be contaminated with pharmaceuticals, the source of which is, for example, treated wastewater that is used to irrigate plants. A number of studies have already proven that plants have the ability to absorb, accumulate and transform certain compounds. It depends on a number of parameters, for example the distribution of roots and compounds in the soil. The distribution of substances in the soil is determined by their sorption on soil particles, transport with flowing water, and their transformation in the soil environment. Compounds that are more sorbed to the soil are less mobile and remain near the soil surface after irrigation. On the contrary, substances that are sorbed less penetrate to greater depths. Thus, the size of the containers can affect the uptake of compounds by plants. This study therefore focused on comparison of the results obtained for pea plants (*Pisum sativum*, L.), which were grown in containers with a volume of 340-cm³ and in cylinders with a volume of 1185-cm³. The plants were irrigated with a mixture of 6 pharmaceuticals: carbamazepine (CAR), citalopram (CIT), clindamycin (CLI), fexofenadine (FEX), irbesartan (IRB), and sulfamethoxazole (SUL). During both experiments, the concentrations of these pharmaceuticals and some of their metabolites were analyzed both in soil and in plant tissues. The absorption of compounds and their distribution in plant tissues partially corresponded to the assumptions based on the dissociation of substances. While the concentration in the roots corresponded to a certain extent to the concentrations in the soil, CAR and its metabolites were mainly present in the above ground plant parts. The content of substances in plant tissues was greatly influenced by the ability of plants to transform substances. The pods contained more metabolites than the primary substances CAR, CLI and SUL. Bioaccumulation factors found for 340-cm³ pots were mostly greater than those obtained for 1185-cm³ cylinders, except SUL. The values were influenced by the distribution of the roots, the availability of compounds for plants and in some cases (e.g. SUL) probably also the different rate of degradation of substances in the soil.

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3.20.P-We293 Pesticides in Pollen and Nectar: Initial Residue Concentrations and Their Dependency on Chemical, Application and Plant Characteristics

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A severe decline of insect biomass has been observed in Europe in recent years, and honeybees were affected by colony collapse disorder. The application of pesticides was identified as one of the major factors for this development, and guidelines for risk assessment of plant protection products on honeybees, bumble bees and solitary bees were developed aiming at minimizing impacts and risks. We have collected a large data set on pesticide residue levels observed in pollen, nectar and other plant parts potentially exposing pollinators. In total, 1720 data were retrieved from EFSA (European Food Safety Authority)

documents and scientific literature, covering 71 different chemicals and 20 crops. From this data set, initial concentrations for pollen and nectar arising from spray application were selected, measured at the time of application up to one day later. These initial concentrations were referred to the application rate, yielding residue per unit dose (RUD) data sets with 161 events for pollen with 32 different chemicals and 164 events for nectar with 27 different chemicals. Median RUD values were calculated to describe the typical realistic initial applied dose.

Earlier findings from risk assessment of plant protection products on bees can overall be confirmed, where RUD in pollen exceeds RUD in nectar and results show a wide variability. Polar pesticides had higher median RUD values, especially in nectar, but the explained variability of substance properties is generally small (10% or lower). Experimental setup and conditions were found to override substance-specific differences. We identified the following factors that contribute to the observed differences in RUD: spray direction (up/sideward > downward), sampling method of nectar and pollen (from flower > by traps > from bees), experimental setting (greenhouse > field or tunnel) and geometry of flowers (Fabaceae had the highest RUD in nectar).

One goal of this study was to find a prediction method for initial pesticide residues in nectar and pollen after spray application to flowers and plants. We have provided median RUD values for the different conditions. Physicochemical properties of the active ingredients revealed some but no dominant influence on the RUD nectar and pollen. Currently, no conclusions can be drawn on how combinations of the mentioned factors affect RUD variability.

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3.20.P-We294 Insecticide Uptake and Fate in Pepper and Tomato Plants – Experiments and Modeling Considering Different Plant Growth Assumptions

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Agricultural activities are intensified globally, often accompanied by an increasing use of agrochemicals like pesticides, which can pose significant risks to ecosystems and human health. We have carried out field and greenhouse experiments with pepper and tomato plants and six frequently used insecticides. Those included acetamiprid, indoxacarb, deltamethrin, γ -cyhalothrin, spinosad and chlorantraniliprole, applied via spray as individual compounds to different experimental plots. Observations were interpreted with numerical modeling, in order to identify and quantify relevant fate processes.

The observed dynamics of fruit concentrations could be described overall well by modeling. After application, concentrations decreased in pepper and tomato fruits, where lower degradation and dissipation was observed for tomatoes. Chemical input to individual above-ground compartments (fruit, leaf, stem) and soil, arising from spray, was among the unknowns and therefore required to be estimated. Input to fruits was estimated 1-13% and 1-17% of the total applied amount, and input to stem, leaf and/or soil 0-13% and 0-26% (ranges for pepper and tomato, respectively). Estimated chemical input was revealed to be highly variable across the investigated compounds, with considerable uncertainty due to a partly low sensitivity of stem/leaf/soil input to fruit concentrations. The pathway stem-fruit was relevant for all compounds except γ -cyhalothrin (both for pepper and tomato) and deltamethrin (for tomato). The pathways soil-root-stem-fruit and leaf-stem-fruit (phloem) were only sensitive for acetamiprid and chlorantraniliprole.

Our developed dynamic model approach, implementing the appearance and growth of individual fruits, was after calibration successful in describing insecticide fate in pepper and tomato plants. One focus of our investigation was on dynamic modelling of plant growth and connected xylem and phloem flow, where the dynamic approach revealed superior to the assumption of constant plant mass and transpiration, combined with first-order rate constants for growth dilution. Information on the time-window of experiments within the vegetation period and on the number and appearance of individual fruits is important for adequately describing growth and thus chemical fate within plants. The model can readily be applied for other compounds and plants, if related data on chemical properties and plant characteristics are available.

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3.20.P-We295 Phosphorus Mobility in Oxisols: Implications of Phosphorus Sources for Sustainable Agricultural Systems

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A comprehensive understanding of the interrelationship between soil texture, pH, and phosphorus (P) sources is crucial for the advancement of agricultural practices and the reduction of environmental impacts. The objective of this study was to investigate the influence of soil texture and phosphorus (P) sources on phosphorus mobility in two Oxisols with contrasting clay contents: a sandy loam and a clay soil. The movement of phosphorus derived from organomineral fertilisers and triple superphosphate was assessed using miscible displacement experiments conducted in acrylic soil columns. The experiments were conducted under two pH conditions: 4.8 (natural) and 6.0 (adjusted). Breakthrough curve (BTC) analysis demonstrated that at a natural pH of 4.8, the two-site kinetic model provided an effective description of P dynamics, capturing the complex interactions between phosphorus ions and the soil matrix. In contrast, at pH 6.0, a linear model proved to be a superior representation of the simplified P transport mechanisms. The results demonstrated that clay-rich soils exhibited a greater phosphorus sorption capacity, particularly at lower pH levels, while sandy loam soils demonstrated a higher risk of nutrient leaching due to rapid phosphorus movement. Furthermore, the findings emphasized the importance of selecting appropriate phosphorus sources, as these choices significantly influence mobility and availability. This research provides actionable insights for managing phosphorus in highly weathered tropical soils, aiding the development of agricultural strategies that balance soil fertility and environmental protection.

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3.20.P-We296 Seasonal Variation in Uptake of Contaminants of Emerging Concern into Edible Plants: Insights from a Field Study from Australia

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Irrigation of agricultural crops with recycled wastewater is becoming an increasingly important means of ensuring consistent access of water in Australia. There is growing concern that contaminants in recycled water may affect edible crops. Accordingly, the aims of the project were to: 1) Detect and quantify >1000 contaminants of emerging concern in recycled wastewater (class A,C), soil and plant tissues used for crop irrigation; 2) Quantify contaminants in roots, leaves and florets to unravel uptake and distribution within plant tissues under field conditions over two consecutive years; 3) Measure indicators of plant condition (e.g. physiological traits) to assess whether recycled wastewater has an adverse impact on crop health. Plant, soil and water samples were collected from five farms using recycled water and from two reference farms (not using recycled wastewater). Water, soil and plant tissues were screened for trace metals and >1000 organic chemicals (pesticides, PPCPs, EDCs, PFAS and a suite of industrial chemicals) along with nine genes related to antimicrobial resistance (AMR). Targeted, quantitative analysis (LC-MS/MS) was used for organic chemicals, along with suspect screening on high resolution mass spectrometry (HRMS) analysis. A total of 143 samples was collected, of which 41 were plants (roots, shoot, florets), 41 soil, and 55 water samples. Our study shows that of the targeted organic contaminants, >65 were measured in water and 100 in plants, with the majority being pesticides, as well as PPCPs, industrial chemicals, PFAS and phthalates, and typically at low (ng/L) concentrations. The majority of these chemicals, apart from PPCPs and EDCs, were also measured at reference sites, suggesting additional sources of contamination contributed to the residues detected in recycled water. Suspect screening revealed additional contaminants not included in the targeted quantitative suite, with significantly elevated concentrations in wastewater compared to surface water. Most of these contaminants were identified as pesticides and PPCPs. Seasonality differences were detected with lower overall concentrations of contaminants detected in samples collected in 2024 vs 2023. Ongoing analysis of HRMS data will assess whether potentially hazardous chemical residues were taken up in edible plant tissues from wastewater irrigation. Overall, our

study shows that wastewater irrigation in these scenarios is unlikely to cause harm to consumers or adjacent environments.

3.20.P-We297 A Monitoring Study to Ascertain a Baseline for Organic Contaminants in UK Agricultural Soils

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The application of organic wastes, such as animal manures and biosolids, as soil amendments (animal manures and biosolids) is a common practice within the UK. Such practices are known to improve soil fertility from a nutrient perspective but they are also well known to introduce emerging contaminants into terrestrial ecosystems. This raises significant environmental and human health concerns associated with their translocation to receiving aquatic compartments but also for uptake into arable crop (produce for consumption). The extent of emerging organic contaminants in UK soils is not comprehensively understood. To address this knowledge gap sixteen farms (30 fields) across the UK were sampled in 2022 (<200 field samples). A validated, multi-residue extraction technique (n = 19, 0.1–30 µg/kg) was employed on the soil samples prior to analysis via non-target approaches (HRMS) and targeted approaches (LCMS/MS). The target chemicals included a diverse array of organic contaminants, including human pharmaceuticals (e.g., antibiotics, anticonvulsants, NSAIDs), veterinary medicines, personal care products, herbicides, pesticides, and biocides. Our findings reveal the presence of several organic contaminants across the sampled farms, including carbamazepine (0.03–44.49 µg/kg dw), clotrimazole (0.2–274 µg/kg dw), oxytetracycline (12.25–29.24 µg/kg dw), enrofloxacin (0.15–9.58 µg/kg dw), and diazinon (2.96–7.19 µg/kg dw), while metformin and ofloxacin were identified to be <LOQ. Notably, non-target screening identified over 300 additional organic contaminants in soil extracts (pharmaceuticals>plasticizers>research chemicals>fungicides>veterinary medicines). The data indicate higher levels of contamination in soils treated with biosolids compared to those receiving conventional animal manures. These results highlight the significant impact of utilizing biosolids and underscore the nascent stage of our understanding regarding the risks associated with these practices. Given the projected increase in biosolid use as part of sustainability goals, there is an urgent need for more comprehensive risk evaluations to better assess the long-term implications of persistent chemical contaminants in soils and their potential effects on the food chain.

3.20.P-We298 Understanding Movement of Dimethylsilanediol (DMSD) in Soil: In situ Transformation and Transport Behaviors

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As polydimethylsiloxanes (PDMS) and volatile methylsiloxanes (VMS) are widely used, understanding the fate and distribution of their degradation products in the environment is essential for assessing their overall fate and exposure to humans and ecosystems. During their production and uses, PDMS and VMS may enter wastewater treatment plants and then partition to sludge. If biosolids are applied to agricultural soil as fertilizer, PDMS and VMS may be deposited in the topsoil and begin to degrade. Degradation products form at varying rates depending on soil conditions. Dimethylsilanediol (DMSD), a major degradation product of xxx, undergoes various fate processes, including volatilization, phytovolatilization, degradation (biotic and abiotic), soil runoff, and vertical migration. Understanding the fate and transport of DMSD in soil is needed for evaluating the ultimate fate of VMS. Recent experimental studies on the fate of DMSD in bare soil and plant-soil systems have significantly advanced our understanding of the vertical movement of DMSD in soil. In this study, we developed mathematical models and optimized the parameters with experimental data to better understand i) upward transport of DMSD to topsoil along with pore water during dry periods, ii) volatilization into the air at the soil surface, and iii) phytovolatilization via plant transpiration. Our model predicted DMSD concentrations and movements that were in good agreement with measured data when DMSD was directly spiked in topsoil. Additionally, we applied the model across various scenarios to examine how key parameters influence the transport of water and DMSD in both bare soil and plant-soil systems. Sensitivity analyses show that soil type, KOC and KOA are key factors governing hydraulic conductivity, DMSD versus water transport and rate of DMSD transfer to air. Furthermore, we improved the model by incorporating in situ formation of DMSD from its precursors with no direct addition of DMSD and tested different environmental variables to investigate the transport of DMSD in soil columns. The model predicts that DMSD would be effectively removed from the topsoil layer, with negligible downward movement to deeper soil layers. DMSD accumulation is not expected even after multiple soil amendments by land application of wastewater biosolids. This research

enhances our scientific understanding of the dynamic transport of DMSD and water, both in the presence and absence of plants.

3.20.P-We299 Immobilization and Geochemical Fractionation of Metalloids in Contaminated Soils Amended with Waste-Based Sorbents

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Immobilization of metalloids in soils by sorbents is one of the means how to reduce their bioavailability. Metalloids sorb to mineral and organic particles through various mechanisms that influence their geochemical lability. The knowledge about immobilization potential of sorbents together with geochemical fractionation of arsenic (As) and antimony (Sb) is therefore essential in the process of risk assessment. In this work, immobilization of As and Sb in contaminated soils from mining site located near town Pezinok, in Western Slovakia by waste-based amendments was investigated. Exhausted coffee and black tea waste, compost, hard wood and sewage sludge biochar, iron scrap, non-stabilized iron sludge and iron sludge neutralized with calcium hydroxide were mixed with contaminated soils and incubated at laboratory conditions. The efficiency of amendments was evaluated by the comparison of As and Sb concentrations in demineralized water extracts. The geochemical fractionation of metalloids in amended soils was assessed using sequential extraction procedure. Ammonium sulphate, ammonium phosphate, oxalate buffer with oxalic acid and oxalate buffer with ascorbic acid were used to extract non-specifically sorbed fraction, specifically sorbed fraction, fractions bound to amorphous and crystallized Fe/Al oxyhydroxides, respectively. The results showed that iron scrap and non-stabilized iron sludge were the most effective immobilizing amendments and reduced the concentration of metalloids in demineralized water and ammonium sulphate extracts when compared with non-amended soils. Organic based amendments and neutralized iron sludge showed either no effect on metalloids mobility or, on the contrary, they increased the concentration of As and Sb in the most bioavailable fractions. The lowest concentrations of metalloids in specifically bound fractions were found in iron scrap amended soils. The results of sequential extraction also showed that As was more prone to displacement from soil sorption complex by phosphate than Sb. Amorphous Fe/Al oxyhydroxides were the main scavengers of As, while Sb was immobilized by both amorphous and crystallized phases of Fe/Al oxyhydroxides. In conclusion, Fe-based waste soil amendments were able to immobilize metalloids in contaminated soils and reduced bioavailable fractions of As and Sb in the extracts. Further research is needed to verify the efficiency of the sorbents in large scale and field conditions.

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3.20.P-We300 Wide-Scope Screening of Over 2000 Micropollutants and Transformation Products in Agricultural Soils under Long-Term Reclaimed Water Irrigation

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Considering the large number of micropollutants commonly found in reclaimed water and their various physicochemical properties, it is necessary to apply wide-scope analytical methodologies to provide a thorough evaluation and, therefore, a better understanding of their fate in soil when reclaimed water is used for irrigation. In this study, we analyzed the occurrence of >2000 micropollutants, including transformation products (TPs), in agricultural soils collected in July 2020 from reclaimed water-irrigated fields with different irrigation histories (i.e., increasing irrigation periods from 0.5 to 10 years). The

sampled fields, located in the suburbs of Nicosia in Cyprus, were cultivated with alfalfa and were irrigated with reclaimed water from a membrane bioreactor (MBR) treatment facility. To the authors' knowledge, this is one of the very first studies where the occurrence of such a large number of micropollutants belonging to various chemical classes (i.e., pesticides, pharmaceuticals, drugs of abuse, industrial chemicals, TPs, etc.) with different physicochemical properties is investigated in long-term reclaimed water-irrigated fields. A generic sample preparation method using solid-liquid extraction followed by solid-phase extraction was applied. The extracts were analyzed by ultra-high performance liquid chromatography coupled with a quadrupole time-of-flight mass spectrometer. Target analysis was performed with the use of an in-house developed database of 2466 micropollutants, including TPs. In total, 32 compounds were detected in the soil samples belonging mostly to pharmaceuticals, TPs of pharmaceuticals, and pesticides. Specifically, our analysis revealed the presence of 28 pharmaceuticals and TPs of pharmaceuticals, along with three pesticides and caffeine in the soil samples. Among these, 10 compounds, namely caffeine, carbamazepine, clarithromycin, climbazole, fenbendazole, flumazenil, lamotrigine, telmisartan, tramadol, and venlafaxine, exhibited a frequency of appearance exceeding 70%. It should be also highlighted that 3 TPs of 2 corresponding parent compounds (i.e., omeprazole and venlafaxine) were also detected in the soil samples. The results indicate that attention should be paid to agricultural soils irrigated with reclaimed water not only from the point of view of the physicochemical characteristics of reclaimed water but also from the point of view of the concentration levels of micropollutants and their potential accumulation.

3.20.P-We301 Establishing Ecosystem Stress Baselines Using Drone-Based Multispectral Imaging: Vegetation Indices and Seasonal Dynamics in Plant Communities

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Effective diagnosis of ecosystem stress in terrestrial ecosystems requires reliable baseline data that reflect natural variability in vegetation health. This study aims to establish diagnostic thresholds for vegetation stress in Korean vegetation communities by analyzing baseline vegetation indices and seasonal dynamics using drone-based multispectral imaging. A key challenge is the lack of sufficient baseline data under normal conditions for identifying and comparing ecological damage, as well as distinguishing natural variability from stress-induced changes in vegetation indices such as the Normalized Difference Vegetation Index (NDVI), Normalized Difference Red Edge Index (NDRE), and Green Normalized Difference Vegetation Index (GNDVI). To address these challenges, baseline data were collected from coniferous and broadleaf vegetation communities under normal conditions using drone-based multispectral imaging. From these data, mean values and standard deviations were calculated, and 95% confidence intervals were established as potential diagnostic thresholds. Seasonal variability in vegetation indices was also examined to determine natural patterns, which can provide a framework for evaluating potential stressors, such as chemical exposure. Comparative analysis revealed distinct seasonal dynamics and baseline differences between coniferous and broadleaf communities, influenced by leaf presence or absence. General trends in vegetation indices were consistent across years, indicating that these indices can effectively capture seasonal changes and provide meaningful insights into vegetation health. The findings suggest potential for developing specialized diagnostic models tailored to different vegetation types, advancing ecosystem stress detection and monitoring. The integration of high-resolution drone-based multispectral imaging and reliable baseline data enhances the ability to monitor large areas with greater accuracy compared to traditional satellite imagery or manual inspections. This research lays the foundation for ecotoxicological diagnostic tools and strengthens environmental monitoring applications by integrating drone-based imaging with established baselines.

3.20.P-We302 PFAS Mixtures in Irrigation Ponds: Effects on Soybean (*Glycine max*) Reproductive Phenotype

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As climate change intensifies droughts, irrigation ponds are becoming essential for agricultural water supply. However, these ponds may be vulnerable to contamination by per- and polyfluoroalkyl substances (PFAS) through runoff from biosolid-amended fields. Understanding how runoff influences PFAS levels in ponds and their impacts on crops is critical for sustainable agriculture. This study examined PFAS contamination in an agricultural system with biosolid application. Samples from irrigation ponds, runoff ditches, and soil were analyzed, revealing 12 PFAS compounds in runoff ditches. Perfluorooctanoic acid

(PFOA) and perfluorobutane sulfonate (PFBS) were most abundant (60 ± 4 ppt and 78 ± 11 ppt, respectively). Short-chain PFAS totaled 211.4 ppt, exceeding long-chain PFAS at 90.2 ppt. Irrigation pond water contained similar PFAS, but at six times lower concentrations (48.86 ppt). Soil exhibited the highest total PFAS levels (6247 ppt), dominated by long-chain PFAS (5644 ppt). A growth chamber experiment evaluated the effects of PFAS mixtures (25, 250, 2500, and 25000 ppt) on soybean plants over 150 days. Plant height, chlorophyll content, pod and bean production, biochemical responses, and PFAS accumulation were measured. PFAS uptake followed a chain-length-dependent mechanism: short-chain PFAS (PFBA, PFPeA, PFBS, PFHxA) were detected in beans at 250 ppt, while long-chain PFAS (PFHpA, PFHpS) appeared only at 25000 ppt alongside short-chain PFAS. High PFAS concentrations reduced shoot height during early growth, though recovery occurred by Day 50. Chlorophyll content increased at Day 15, reappearing in the highest treatments by Day 50. Bean production and nutritional quality were modulated by PFAS exposure, with the 25000 ppt group producing three times as many beans (14 per plant) as the control (4 per plant, $p < 0.01$). Water availability improved soybean resilience under PFAS stress, supporting recovery and enhanced reproduction. Biochemical analyses indicated oxidative stress, with elevated ROS, SOD, and CAT levels at high PFAS concentrations. Root transcriptomics revealed upregulation of water uptake pathways and aquaporin genes, alongside downregulation of biosynthetic pathways, likely reallocating resources to reproduction. These findings highlight the risks of PFAS in irrigation systems and their chain-length-dependent accumulation in edible tissues.

3.20.P-We303 Does PFAS Pollution Result in Smaller Beans? A Case Study on PFAS Accumulation and Growth Hormone Levels in Broad Beans

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Per- and polyfluoroalkyl substances (PFAS) are widely used, resulting in substantial accumulation and pollution by these forever chemicals. Terrestrial plants are able to take up PFAS through their roots, and previous research suggested an association between PFAS accumulation in plants and their phytohormone levels.

In this study we focused on broad bean plants (*Vicia faba* L) and compared PFAS bioaccumulation, translocation, and distribution patterns with phytohormone levels in various plant parts. The targeted hormones included aminocyclopropane-1-carboxylic acid, cytokinins, indole-3-acetic acid, gibberellins, jasmonic acid, and abscisic acid, along with the amino acid methionine, which is essential for ethylene biosynthesis.

An environmentally relevant PFAS mixture, containing the ten most dominant PFAS present in private gardens near a PFAS hotspot, was used in this study. These compounds with varying chain length and functional groups were chosen to identify potential distribution differences within the plant due to the characteristics of these individual compounds. In a greenhouse set-up, individual plants were grown on soil spiked with three different concentrations of this PFAS mixture alongside a control group. The bean plants were separated into two harvest groups whereby plants were harvested when the plants entered the flowering stage (40 days of exposure), whereas other plants were harvested after pod and seed development (70 days of exposure). Upper leaves (above the flowers), middle leaves (flower zone), lower leaves (below the flowers), flowers, apex, middle stem, lower stem, roots, beans and pods of *V. faba* were collected separately and flash frozen in liquid nitrogen.

After solvent extraction and solid phase extraction, PFAS and phytohormones were quantified using UPLC-MS/MS. Image analysis of each individual plant was conducted using Image J to identify differences in growth. The results will identify bioaccumulation, translocation and distribution patterns of the 10 PFAS compounds added to the broad bean plant. Comparing the PFAS-related results and size of the different plant parts to the hormone levels in each plant part, will allow us to understand how PFAS affects plant growth and which mechanisms are involved.

In addition, this study will identify PFAS accumulation sites within this species, which is crucial for assessing potential human consumption risks.

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3.20.P-We304 Bioaccumulation of Legacy and Novel Per- and Polyfluoroalkyl Substances (PFAS) in Food Plants

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Per- and polyfluoroalkyl substances (PFAS), known as forever chemicals, are a class of fluorinated organic compounds commonly used in industrial and consumer products worldwide. After decades of use, PFAS are ubiquitous in environmental matrices, including in ground and surface water, wastewater, soil, sediment, and biota. The persistence, bioaccumulative potential, and toxicity of legacy PFAS have led to global restrictions of some compounds such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), prompting the use of novel alternatives. The environmental fate and exposure risks of such alternatives are not fully understood, though some studies suggest toxicities comparable to their legacy counterparts. Industrial and domestic emissions are the main contributors to environmental PFAS contamination, while practices such as irrigation with reclaimed water and biosolids application may further introduce PFAS to agricultural systems, and thus, the food chain. Crop accumulation and subsequent human consumption is a significant pathway of dietary exposure to PFAS. Therefore, the behavior of PFAS, and especially the novel alternatives, in food plants requires urgent attention. One class of novel alternatives is ether-PFAS, which contain one or more oxygen atoms in the carbon backbone. The goal of this study was to investigate the extent of plant biotransformation of ether-PFAS and characterize the transformation products for the first time. To achieve this, three representative ether-PFAS compounds were selected: hexafluoropropylene oxide dimer acid (HFPO-DA or GenX), 4,8-dioxa-3H-perfluorononanoic acid (ADONA), and 6:2 chlorinated polyfluorinated ether sulfonic acid (6:2 Cl-PFESA). The parent compounds were spiked individually into *Arabidopsis thaliana* (thale cress) cell cultures, which were sampled after 0, 3, 6, 12, 24, 48, and 96 h of incubation. Parent compound concentrations were determined for each sampling time using UPLC-MS/MS, and statistically significant differences in concentrations between compounds and sampling times were evaluated using one-way ANOVA and Tukey's HSD ($p < 0.05$). A non-targeted screening and profiling of the biotransformation products was conducted using HPLC QTOF-MS, with product identities confirmed against authentic standards. Overall, this study provides novel insight into the behavior and fate of ether-PFAS in plants, which is essential for future ecosystem and human health risk assessments.

3.21 Understanding the Complexity of Tire Particles and Associated Chemicals: Environmental Monitoring, Toxicological Effects and Strategies for Mitigation

3.21.T-01 Leaching of Organic Compounds from Tire Particles Under Conditions Simulating the Deep Sea

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Tires can contain high amounts additive chemicals, which can leach out and enter the (aquatic) environment, with potential toxic consequences. Little is known on the impact of high hydrostatic pressure on anthropogenic particles and leaching of chemicals. Therefore, our objective was to investigate the leaching of organic compounds from tire particles in natural seawater under atmospheric pressure vs. high hydrostatic pressure conditions. The test material consisted of (i) cryo-milled tire thread (CMTT), (ii) virgin crumb rubber (VCR) from an artificial soccer field and (iii) weathered crumb rubber (WCR) which consisted of VCR that had been immersed in the sea for 12-18 months prior to the experiments. Seawater was collected at the surface and at 2000 m depth, filtered (0.7 µm GF/F) and filled into glass bottles containing the test material. The samples were then subjected to atmospheric pressure and high hydrostatic pressure (200 bar) conditions, respectively. A subset of abiotic samples was made for both pressure conditions by adding HgCl₂ (final conc. 10 mg L⁻¹). Samples were taken after 6 h, 24 h, 7 days and 14 days, filtered and analyzed for DOC, nutrients, prokaryotic abundance and organic compounds. High hydrostatic pressure was shown to influence bacterial growth, particle agglomeration and chemical leaching of tire particles. CMTT leached higher amounts of chemicals, while WCR released phosphate from its former biofilm into the dissolved water phase, thereby further boosting bacterial growth. Leaching of target analytes was generally higher under high hydrostatic pressure conditions and under biotic conditions, with opposite trends concerning the latter for chemicals prone to biotransformation processes. Interestingly, even after 12 months in the environment WCR still released a variety of organic compounds, suggesting a long-term potential of tire particles to release additive compounds into the marine environment.

3.21.T-02 Stability Assessment of Tire-Derived Chemicals for Quantitative Air Monitoring with Passive Samplers and Filters

Raimon M. Prats¹, Alexander Kasperkiewicz¹, Amandeep Saini², Chubashini Shunthirasingham¹ and Tom Harner², (1)Environment and Climate Change Canada (ECCC), Canada, (2)Environment and Climate Change Canada (ECCC), Gatineau, Canada Tire-derived chemicals (TDCs) are a diverse group of organic pollutants found in the complex chemical mixture that makes up vehicle tires. Many of these compounds (e.g., phenylenediamines like 6PPD, benzothiazoles and benzotriazoles) serve as rubber additives, acting as vulcanization agents, antioxidants, or UV-stabilizers. This broad group of environmental contaminants has recently gained prominence due to reports of acute toxicity and their potential to generate atmospheric transformation products that present additional environmental and health risks.

Recent studies have shown that TDCs are ubiquitous in urban air globally and demonstrated that passive air sampling (PAS) techniques can effectively track and monitor TDCs and derived products in air across major cities worldwide. However, studies are often constrained to qualitative or semi-quantitative characterizations of TDCs due to the uncertainty posed by their rapid degradation.

Thus, there is a need for a comprehensive assessment of TDC stability in collected samples and solvent extracts to reduce uncertainties and enable a more confident and quantitative approach to TDC analysis. We present preliminary findings from a degradation study encompassing over 50 native TDCs, TDC derivatives, and other chemical groups. The stability of these compounds was evaluated in various organic solvents, in processed and stored sample extracts, and under sampler storage conditions for polyurethane foam (PUF) PAS and glass fibre filter air samples.

Results indicate that most TDCs dissolved in solvents remain stable for a few days after spiking, with methanol showing the lowest analytical variability. However, some compounds, like 77PD in methanol, experienced steep declines, dropping to 7% of their initial levels, while IPPD showed moderate decreases after three days. Storage temperature had no noticeable effect on stability. In contrast, significant reductions in relative concentrations were observed for several TDCs in stored sampler extracts for 50 days, with declines ranging from 15% to 50% for many compounds and up to 80% for chemicals like 6PPD and CPPD.

These findings highlight the need for optimized protocols to minimize degradation or account for its magnitude, particularly in long-term stored sampler extracts that may experience more significant declines in concentrations for certain compounds.

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3.21.T-03 Tyre Particles: Who Dares Win. A Comparison Study Evaluating the Sensitivity of Two Marine Invertebrates to Whole Tyre Particle Toxicity

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Tyre particles are an anthropogenic pollutant of high environmental concern. Estuaries have been identified as potential sink for these particles owing to the influx of particulates from upstream sources, highway drainage and overlying bridges. To date, research has primarily focused on understanding the effects of tyre particles on freshwater organisms, however, it is important that we have toxicological research to understand how these particles affect biota across affected habitats. In this study, two estuarine invertebrates (*Corophium volutator* and *Marinogammarus marinus*) were selected to assess the ecotoxicological effects of tyre particles; 20, 40, 60, 80, 100, 120 g/kg (dw) sediment, with the addition of a control containing 0 g/kg (dw) sediment and a positive control, 3,5-Dichlorophenol (3,5-DCP). It is hypothesised that with increasing concentration of tyre particles, there will be an increased risk to biota, with increased mortality and negative impacts on indicators of health. *C. volutator* were taken from laboratory stocks and *M. marinus* were collected from a clean field site and acclimated for 7 days in holding aquaria. Cohorts of four individuals (for each species) were added to a 1 L beaker containing estuarine sediments enriched with tyre particles for a duration of 10 days. During the exposure, mortality, burial data (*C. volutator*) and growth (*M. marinus*) were recorded at regular intervals. Preliminary results show that tyre particles enhanced mortality in both species, with the potential to negatively affect food webs leading to cascading effects. Further, *C. volutator* demonstrated diminished burial behaviour when exposed to tyre particles, making these individuals more prone to predation. The outcomes of this study will elucidate the risk tyre particles pose to biota in benthic estuarine and coastal environments.

3.21.T-04 Realistic Stress Scenarios for the Ecotoxicity Evaluation of Tire Wear Particles

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Tire wear particles (TWP) are increasingly recognized as a major source of microplastic pollution in air, water, and soil, with risks posed by both the physical particles and their chemical constituents. Tire treads are complex mixtures of natural and synthetic rubbers, fillers, and additives, which differ between passenger car and truck tires. Ecotoxicological studies have linked TWP to oxidative stress, inflammation, and genotoxic effects in organisms. However, some ecotoxicological studies report no effects for TWP. In this study, we systematically investigated TWP leaching over almost three months under conditions simulating mechanical stress, light, and temperature variations. Using TWP from passenger cars and trucks, the ecotoxicological impact was assessed with algae growth tests, complemented by analytical methods to characterize TWP and their leachates.

TWP were generated using tire buffing process and stressed in microcosms containing demineralized water and artificial sediment for 75 days. Experimental conditions included different TWP treatments (e.g., with/without antioxidants, pre-aged), concentrations (120 mg/l, 600 mg/l), light exposure, temperatures and mechanical abrasion. Aqueous samples, filtered at four intervals, were ecotoxicologically assessed using an algae growth inhibition test. TWP surface changes were analyzed via SEM and EDS and particle composition via Pyr-GC/MS and TGA, alongside leachate chemical analysis. Blank samples promoted slight algae growth, probably due to nutrient leaching from artificial sediment. Filtered samples from tested TWP generally promoted algae growth, varying by conditions and TWP settings. Truck TWP leachates caused lower growth promotion than passenger car TWP, indicating growth promoting leachates from these TWP. In turn, truck TWP exhibited reduced zinc content after weathering, possibly contributing to lower growth promotion. Comparison of antioxidant impacts showed differing effects by passenger car and truck TWP.

Chemical analysis revealed weathering-induced changes, including decreased surface carbon and increased oxygen, silicon, and aluminum signals, indicating surface oxidation or silica/alumina agglomeration.

Overall, stressed TWP did not inhibit algae growth but nutrient supply from sediment and degradation products likely promoted growth or reduced inhibition to baseline levels. This study highlights the role of chemical composition in TWP degradation and leaching.

3.21.P-Th278 Human Exposure Assessment to Tire-Related Chemicals through Urine and Silicone Wristbands: Method Development

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Tire-road wear particles (TRWPs) are generated through tire-road friction and are a significant source of non-exhaust emissions. Their accumulation in the environment raises concerns about human health due to the toxicity of Tire-Related Chemicals (TRCs), which include additives and their transformation products. Human exposure to TRCs occurs primarily through inhalation, ingestion, and dermal contact. This study aims to develop and validate methods for quantifying TRCs in urine and silicone band passive samplers. Urine, commonly used in exposome research, and silicone bands, which mimic dermal exposure, offer insights into skin exposure and internal concentrations.

Sample preparation protocols were optimized for recovery and reduced matrix effects. For silicone bands, the solvent volume, number of extractions, and reconstitution volumes were refined, with a final protocol requiring two 15-minute extractions with methanol-ethyl acetate (1:1 v/v) and reconstitution in methanol-water (1:1 v/v). Urine samples were hydrolyzed with β -glucuronidase to process metabolites, and β -glucuronidase filtration and liquid-liquid extraction (LLE) methods were compared. While β -glucuronidase provided cleaner samples, its dilution limited detection sensitivity. LLE, with 10-fold concentration and internal calibration, allowed the detection of low-sensitivity compounds despite matrix challenges.

Silicone band extraction yielded quantitative recovery for most compounds after two extractions, validated at three concentration levels. For urine samples, LLE enabled the quantification of low-sensitivity compounds despite matrix effects. Preliminary findings on healthy volunteers detected benzothiazoles, benzotriazoles, and cyclic amines in both matrices, with more compounds identified in urine.

These validated methods provide a framework for assessing human exposure to TRCs, identifying biomarkers of TRWPs, and evaluating health risks. By comparing innovative silicone bands with traditional urine sampling, this study enhances understanding of the environmental and human health impacts of tire-related chemicals.

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3.21.P-Th283 Monitoring of Tire-Derived Compounds in Commercial Leafy Vegetables and Associated Dietary Exposure

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The toxicity of tire particles has been linked to leachable organic additives and associated transformation products (tire-derived compounds). Tire particles and tire-derived compounds are introduced to the agricultural environment via atmospheric deposition, irrigation with treated wastewater, and biosolids (treated sewage sludge) application as fertilizer. In the agricultural environment, tire-derived compounds may be taken up by edible plants, leading to human exposure. Sixteen tire-derived compounds were measured in twenty-eight commercial leafy vegetable samples grown in Switzerland, France, Spain, and Israel. In commercial leafy vegetables, six tire-derived compounds were detected: benzothiazole (maximum concentration 238 ng/g dry weight), 2-hydroxybenzothiazole (maximum concentration 665 ng/g dry weight), 1,3-diphenylguanidine (maximum concentration 2.1 ng/g dry weight), N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD, maximum concentration 0.4 ng/g dry weight), N-isopropyl-N-phenyl-4-phenylenediamine (IPPD, maximum concentration 0.1 ng/g dry weight), and N-phenyl-N-cyclohexyl-p-phenylenediamine (CPPD, maximum concentration 0.3 ng/g dry weight). At least one compound was present in 71% of samples analyzed. Statistical analyses did not reveal correlation between known growth conditions and tire-derived compound concentrations in the leafy vegetables. Based on measured concentrations and dietary survey data, the estimated daily intake due to leafy vegetable consumption of these tire-derived compounds under a mean and maximum concentration scenario was calculated. The estimated daily intake for 1,3-diphenylguanidine ranged from 0.05 ng/person/day in the mean scenario to 4.0 ng/person/day in the maximum scenario; benzothiazole ranged from 12 to 1,296 ng/person/day; 6PPD ranged from 0.06 to 2.6 ng/person/day; IPPD ranged from 0.04 to 1.1 ng/person/day; CPPD ranged from 0.05 to 2.6 ng/person/day. The estimated daily intake via leafy vegetable consumption was generally lower than or comparable to exposure via other known sources, although leafy vegetables represent only a fraction of total dietary exposure. Future studies should elucidate pathways of tire-derived compounds from road to field, assess the exposure to transformation products, and investigate the biological effects associated with this exposure.

3.21.P-Th295 A Broad Approach to Understand How 6PPD-q, Combined with Phenanthrene, Affects Thermal Tolerance, Hematology, and Omics in Three Salmonid Species

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Because of climate change, urbanization, and hydrological manipulation of water flows, water temperatures in urban environments are increasing, which has implications for freshwater fish that inhabit cooler streams and rivers. An additional stressor that fish in urban settings must cope with is stormwater, a complex chemical mixture, including many chemicals that originate from car tires, including antioxidants and their transformation products, such as 6PPD and 6PPD-quinone, and polycyclic aromatic hydrocarbons (PAHs), such as phenanthrene. Recently, 6PPD-quinone was identified as the cause of urban-runoff mortality syndrome of Coho salmon (*Oncorhynchus kisutch*) in the Pacific Northwest. Since the discovery of 6PPD-q, environmental and toxicological understanding of 6PPD-q has progressed fast and 6PPD-q is now detected ubiquitously in urban aquatic systems, while the mechanism of acute lethality has been linked to disruption of mitochondrial functions. However, we know very little about how other contaminants in stormwater runoff influence the toxicity of 6PPD-quinone. In addition, the effects of 6PPD-q alone, and in combination with other stormwater related chemicals, on thermal tolerance in fish is not known. To fill this knowledge gap, we investigated how exposure to a road-runoff surrogate-mixture of 6PPD-quinone (0.5 µg l⁻¹) and phenanthrene (5 µg l⁻¹) influenced the thermal avoidance behaviour of three salmonid species: brown (*Salmo trutta*), bull (*Salvelinus confluentus*), and rainbow trout (*Oncorhynchus mykiss*). Additionally, we investigated body burden, xenometabolome, gill and liver transcriptomes, and plasma-related parameters. Bull trout exposed to the individual components and the

mixture presented thermal avoidance behaviour at significantly lower temperatures (mean range: 17.7 to 19.1°C) compared to control (20.7 ± 0.4°C). No such difference was observed among brown trout. Interestingly, a 5.7 and 7.3 percentage unit reduction in hematocrit was observed among 6PPD-q and mixture exposed brown trout, respectively. No reduction in hematocrit was observed among bull trout, although their blood was observed to be more viscous than control, suggesting altered hematology. Assays with rainbow trout are in progress. Comparative and species-specific molecular processes and biomarkers are to be investigated.

3.21.P Understanding the Complexity of Tire Particles and Associated Chemicals: Environmental Monitoring, Toxicological Effects and Strategies for Mitigation

3.21.P-Th266 Spatial and Temporal Distribution of Tire Particles and Related Additives in Freshwater Ecosystems: Insights from a Swiss Pilot Study

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Tire and road wear particles (TRWP) are produced during abrasion of tires on road pavement. 61% of all microplastics entering Lake Geneva (Switzerland) are estimated to originate from tire wear. Field measurements suggest that levels of TRWPs decrease between their emission source and the aquatic environment with concentrations of 0.5 1.2 g kg⁻¹ in river sediment and 0.5 5 mg L⁻¹ in river water. Many chemicals are intensively used as vulcanization agents, antioxidants and antiozonants; they can represent several percent of the tire rubber mass. The distribution and fate of TRWPs and associated chemicals in the aquatic environment are still poorly understood. Moreover, the potential toxic impacts of these tire-associated chemicals for aquatic biota vary widely suggesting specific species sensitivity.

This study aimed (i) to assess the presence and distribution of tire-related additives in water and sediment from alpine/perialpine lakes and urban streams in Switzerland (ii) to retrace tire-related additives contamination history in sediment cores from Lake Geneva and (iii) to identify the input sources of tire-related additives. Water and sediment from Swiss lakes (n = 17), sediment from streams in the Lake Geneva catchment (n = 60), suspended particulate matter (n = 4) and sediment cores from Lake Geneva (n = 2) were collected. Sediment and water were extracted and analysed by UPLC-MS/MS for 15 additives and by Py-GC/MS for TRWP. Our results showed that several additives were present in most perialpine lakes with a predominance of DPG, 6PPD-Q, aniline and benzothiazoles in the ng L⁻¹ range. The presence of roads in the close catchment of the lakes was positively correlated with concentrations of tire-related additives. However, traces of tire-related additives were detected in high altitude remote lakes highlighting the possibility of atmospheric deposition of TRWPs or gaseous tire-related additives. The concentrations of tire-related additives in sediment cores showed negative correlation with depth. Two main mechanisms likely play a role: a higher road traffic in the catchment of the sampling point in recent years together with a potential degradation of tire-related additives in the most ancient sediment layers. Our results provide important data for a better understanding of tire-related additives fate in freshwater ecosystems and could be used for future risk assessment and mitigation studies.

3.21.P-Th267 From Tires to Tributaries: Suspect Screening of Rubber-Based Contaminants in the Tagus River (Spain)

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Products derived from plastic and rubber such as tires or other elastomeric products often contain a wide variety of organic additives such as antioxidants, antiozonants, heat protectors or vulcanizing agents. These substances can be released into the environment through processes such as the erosion and leaching of rubber products either during their use or after disposal. Then, pollutants could be mobilized by rainfall and runoff processes to the aquatic environment where they could pose a risk to these ecosystems. The present study performs an environmental screening to investigate the presence of rubber-derived contaminants in surface water samples (n = 89) from the Tagus River basin (Spain). Samples were collected over three consecutive years (2020, 2021, 2022) along the river's entire course at thirteen locations encompassing both urban and rural areas. Surface water samples were processed using a solid phase extraction methodology and analyzed by liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-QTOF HRMS) with ESI⁺ and data independent acquisition mode.

A suspect screening workflow was designed for data processing, including a suspect list that contains 145 rubber additives of different chemical nature, incorporating impurities and transformation products. More than 25 compounds were detected and identified at different confidence levels according to Schymanski's scale. Among these, cyclic and bicyclic amines, melamines, benzothiazoles or benzotriazoles were reported in the analyzed samples. Some of them such as hexa(methoxymethyl)melamine (HMMM), benzothiazole-2-sulfonic acid, benzotriazole and 5-methyl-1H-benzotriazole, were ubiquitous along the Tagus River basin with detection frequencies exceeding 70%. The presence of HMMM was also associated with other widely distributed structural-related impurities co-occurring in minor intensity in the analyzed samples.

These approaches provide valuable preliminary results for evaluating the environmental distribution of persistent anthropogenic pollutants in the aquatic compartment, facilitating the prioritization of specific compounds in future research. Notably, the suspect list developed in this study confirms that rubber-derived compounds are currently present in the Tagus River surface water.

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3.21.P-Th268 Seasonal Variation of Tire and Road Wear Particles in Urban Particulate Matter
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Tire and road wear particles (TRWP) are known to contribute significantly to urban airborne particulate matter (PM). Besides the synthetic or naturally derived polymeric material, these micro- and nanosized particles are known to contain a variety of potentially hazardous chemicals, causing a risk for both the environment and public health.

TRWP have been reported in every environmental compartment, but quantitative data on airborne TRWP are scarce. Absorption properties and microscopic size of TRWP have proven to be a challenge for microspectroscopic techniques traditionally used in environmental microplastics studies. The aim of this study was to utilize novel thermoanalytical methodology for quantification of TRWP in health relevant PM size fractions: particulate matter smaller than 10 µm and 2.5 µm (PM10 and PM2.5, respectively). PM samples were collected with active aerosol samplers. Sampling took place at two sites in Helsinki, Finland, during winter springtime. Sampling sites represent the exposure for TRWP in urban or urban background environments. Airborne tire wear rubbers were quantified by pyrolysis-gas chromatography-mass spectrometry.

Seasonal variation of tire wear rubber derived TRWP mass concentrations in urban PM10 and PM2.5 is discussed. Development and application of robust and straightforward methods for PM monitoring are needed due to the change in non-exhaust particulate matter emissions caused by transition from internal combustion engine to electric vehicles.

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3.21.P-Th269 Tire and Road Wear Particles in Urban Rivers and Coastal Marine Environments
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Tire and road wear particles (TRWP) are a class of microplastic pollutants of increasing concern, particularly within and adjacent to urban areas because of high emission rates and the toxicity of some tire additives. Although TRWP has been documented in urban waterways, much remains to be learned about their concentration and transport dynamics through watersheds and coastal marine systems. Here we present an overview of TRWP found in several rivers and coastal marine environments in Southern California, USA during our group's water column and sediment sampling activities over the past 4 years. River sampling was conducted during dry weather (no runoff) and stormflow conditions using techniques designed to collect samples representative of concentrations across the depth and width of the flow. Coastal marine water column samples were collected at surface, mid-water, and near bottom depths under summer (little-to-no runoff) and winter (recent stormflow) conditions. Sediment samples were collected through a range of grab and coring techniques. Our river results indicate large concentrations and fluxes of

TRWP relative to many other microplastics found in the same samples, with stormflow dominating transport. TRWP was also a dominant class of microplastics across marine environments, with the highest concentrations in benthic estuarine sediment. However, TRWP was also found throughout the marine water column, even at locations > 40 km offshore during the dry season. This work will serve as a foundation for future TRWP monitoring and further investigation into the impact of these particles and their associated constituents on aquatic systems.

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3.21.P-Th270 Identification of Chemical Hazards in Tire Tread with HPTLC-Based Effect-Directed Analysis

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Tire particles can leach a complex mixture of chemicals to the environment including yet unknown substances. Toxicity of these mixtures is often not explained by target chemical analysis. We aim to detect toxic chemicals in tire tread using in vitro bioassays coupled with chromatography, and ultimately identify those chemicals through effect-directed analysis (EDA).

We extracted chemicals from cryogenically milled tire tread (CMTT) obtained from new tires. We applied estrogen (ER) and aryl hydrocarbon receptor (AhR) bioassays in 96-well and high-performance thin-layer chromatography (HPTLC) plates to detect bioactive chemicals. We compared the unknown chemicals to known tire-associated chemicals, and we collected targeted fractions of active chemicals for chemical analysis. Fractions collected from HPTLC for chemical analysis were retested to confirm toxicity was not lost or subject to transformation. We analyzed the fractions with suspect screening and non-targeted LC-HRMS/MS.

CMTT extracts as whole mixtures in 96-well plates and after separation with HPTLC caused strong agonism of ER and AhR. We observed at least two or four HPTLC-bioassay bands were responsible for ER or AhR, respectively. Although HPTLC bands possibly contain multiple bioactive chemicals, the low number of bands indicates that a minority of chemicals in tire tread are driving ER and AhR activation. Early results showed that among common tire-associated chemicals tested in parallel, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was estrogenic. Collection of the unknown CMTT toxicants into discrete fractions was successful as shown by confirming with the HPTLC-ER and -AhR bioassays. Experiments with HPTLC-bioassays and LC-HRMS/MS showed that the estrogenicity was linked to a 6PPD transformation product: 4-hydroxydiphenylamine. Additional unknown ER and AhR active chemicals are under investigation.

Our work reports on progress of identifying toxicants in tire tread with HPTLC-based EDA. Our strategy uses model tire particles, iterative (HPTLC-) bioassays, fractionation, and chemical analysis to prioritize specific-acting toxicants. The use of CMTT at high concentrations supports our goals of toxicant identification by allowing us to work with a concentrated sample of chemicals linked directly to tires. After identification of these chemical hazards, we can begin to evaluate risk through studies targeting environmentally relevant toxicity and exposure scenarios.

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3.21.P-Th271 Degradation Rates and Ageing Effects of UV on Tyre and Road Wear Particles

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Tyre and road wear particles (TRWPs) are suggested to be the largest source of microplastics in the environment with annual global emissions estimated to be between 800-3300 kt. As the most TRWPs are estimated to end up in roadside soils, understanding environmental fate of TRWPs in this compartment is of great importance. An important variable is how fast TRWPs degrade and what effect this may have on the physicochemical properties of the TRWPs.

The aim of this research is to characterise the effect of UV-degradation on TRWPs and calculate the abiotic degradation rate for TRWPs. To achieve this, samples were aged following an accelerated UV-ageing procedure. Four types of materials were used to investigate the impact of various tyre properties: cryomilled tyre tread from premium car tyres, cryomilled end-of-life truck tyres, particles generated from premium car tyres on a road simulator and particles generated from budget car tyres on a road simulator. All materials were fractionated to 50-200 μm to represent the particles that deposit in roadside soils. The rubber content was analysed at various ageing times using thermogravimetric analysis, changes in tyre additives were investigated using direct thermal desorption gas chromatography mass spectrometry and particle size and morphology were characterised using static light scattering and electron microscopy. For all samples, abiotic degradation was observed to proceed through a first order decay. Cryomilled tyre rubber was seen to degrade faster than road simulator samples, potentially due to a shielding effect of the encrusted road wear particles. When corrected for the accelerated ageing, environmental degradation rates were estimated to be between 0.01-0.03 day⁻¹. Particle size analysis showed that particles became smaller with longer ageing times and a fraction of small particles <10 μm was formed, suggesting that both surface degradation and fragmentation play important roles. This was confirmed with electron microscopy where it was also observed that after ageing particles had a much smoother morphology.

We believe this work can aid fate modelling studies by providing input data on both degradation rate and changes in particle size. This will help with understanding the scale of the problem, and developing suitable mitigation strategies.

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3.21.P-Th272 Unveiling the Impact of UV-Driven Advanced Oxidation: A Comprehensive Study on the Physical and Chemical Transformation of Tire-Derived Microplastics in Aquatic Environments

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Tire-derived microplastics have emerged as a pressing environmental issue, with their persistence and complex chemical makeup posing potential risks in aquatic ecosystems. This study explores how UV radiation alters two prevalent forms of tire-derived microplastics: micro rubber chips and crumb rubber, widely used in artificial playgrounds. Samples were exposed to low energy mercury UV light for durations of 1, 3, and 5 days, allowing us to examine both physical degradation and chemical transformations. Our results revealed marked differences in the breakdown of these materials: micro rubber chips and crumb rubber exhibited maximum weight losses of 0.85% and 0.61%, respectively, following five days of UV exposure. The carbonyl index increased to 0.4 for tire chips and 0.5 for crumb rubber, signifying substantial chemical changes over time. Furthermore, total organic carbon (TOC) levels in the leachates escalated, reaching 6.2 ppm for micro rubber chips and 4.5 ppm for crumb rubber, highlighting a steady release of organic compounds as UV exposure continued. These findings illuminate the transformative effects of UV exposure on tire-derived microplastics, emphasizing that UV-driven fragmentation and chemical leaching contribute to the complexity of microplastic pollution in water bodies. This work calls for further research into the identification and behavior of specific leached chemicals through GCMS analysis and heavy metal analysis with the goal of informing effective mitigation strategies. Our study underscores the urgent need for comprehensive measures to address the degradation and containment of tire-derived microplastics, ultimately safeguarding aquatic ecosystems from their far-reaching impacts.

3.21.P-Th273 Influence of Riverine Salt Gradients on the Adsorption of Trace Elements by Tire and Road Wear Particles: Experimental Approach

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Over the past decade, ecotoxicological concerns arose regarding tire wear particles (TWP) and their potential leachates in aquatic environments. As up to 20,000 t/a TWP are estimated to enter German surface waters, TWP have a non-negligible anthropogenic influence.

Despite this, the role of TWP as potential carriers of contaminants, such as trace elements, remains understudied. In our research, we observed the adsorption of heavy metals like Cr, Ni, Zn, Cd, and Pb onto tire and road wear particles (TRWP) in low-salinity surface water samples. This adsorption could deteriorate the chemical water quality of suspended matter, as classified by the German Working Group of the Federal States on Water Issues.

It is known that all major rivers discharging into the sea experience a salt gradient influencing the adsorption behavior of trace elements onto particles. Therefore, the aim of this study was to investigate how varying salt concentrations in surface waters affect the binding behavior of trace elements on TRWP.

The basic experiment we established for the adsorption of heavy metals onto TRWP is the following: A filtered (0.2 µm) river water sample (Freiberger Mulde, Saxony) was prepared. NaCl was added to obtain the required salt concentration (six samples ranging from 0.15 g/L (Freiberger Mulde) to 15.2 g/L Cl⁻ (tidal Elbe, North Sea)) representing river conditions in the Elbe catchment area. TRWP were added at a concentration of 16.7 mg/L. The samples were shaken for 24 h. After filtration and drying in a desiccator, the particles were digested using HNO₃ and HCl (3:1) and the element content quantified using ICP-MS/MS.

Among the priority elements (Cr, Ni, Cu, Zn, As, Cd, Pb), only the adsorption of Cd and Zn showed a significant dependency on the salt concentration. As Cl⁻ concentration (and thus conductivity) increased, the adsorption capacity of Cd and Zn onto TRWP decreased. This is likely due to the formation of negatively charged chloride complexes [MCl₂]⁻, which do not readily bind to the negatively charged TRWP surface. While cations may form ionic interactions with TRWP, chloride complexes do not. These findings help predict the binding properties of trace elements onto TRWP in environmentally relevant contexts, e.g., such as large rivers systems like the Elbe or in water retention projects like sponge cities. This knowledge can help assess the potential risks posed by trace elements adsorbed onto TRWP.

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3.21.P-Th274 Elastomers or Zinc? A Comparison Between Elemental and Organic Analysis for the Detection and Quantification of TWPs

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Particles produced from tyre wear contribute significantly to the overall plastic pollution of the environment. While a number of different techniques have been utilized in an attempt to provide evidence for the occurrence and fate of tyre wear particles (TWPs) in the environment, data are still relatively scarce when compared to other areas such as microplastics. Thermogravimetric analysis coupled with gas chromatography-mass spectrometry (TGA-GC/MS) provides a method whereby TWPs can be quantified in a number of different environmental matrices with little to no sample preparation. This work will, for the first time, compare the tyre elastomer concentrations found using TGA-GC/MS, with the concentrations of a known elemental tyre wear marker, zinc, in stormwater drainage sediments, and roadside surface soils. ZnO is added as an activator in the vulcanisation stages and comprises approximately 1 to 2% of the tyre tread mass. Quantification of zinc has been used as a method by which to quantify the presence of TWPs. However, in matrices such as sediments where rain and river water are also factors, leaching of zinc may prove problematic for accurate and reliable quantification of TWPs using this element as a marker. In this work, we explore the correlation between zinc levels and the concentrations of styrene-butadiene rubber (SBR) and polyisoprene (PI) found in a number of sediments collected from a drainage facility near the A34 in Newbury (UK) which prevents chemical contaminants from entering an SSSI (site of special scientific interest) river system (Lambourn River), to better understand the efficacy of each marker for the quantification of TWPs. Here, we show that the correlation between elastomer content and zinc concentration is far greater during summer months (R-squared values of 0.99 and 0.93 against SBR and PI respectively) compared to winter months (R-squared values of 0.20 and 0.10) against SBR and PI respectively. Furthermore, we explore the transportation of TWPs throughout a roadside drainage facility, evaluating its efficiency in preventing tyre-derived pollutants from being further distributed into the wider environment.

3.21.P-Th275 Influence of Repeated Leaching of Cryogenically Milled Tyre Tread (CMTT) and Tyre and Road Wear Particles (TRWP) on the Dissolved Concentration of Tyre Derived Chemicals

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Tyre and road wear particles (TRWP) contain ca. 10% organic compounds added to improve tyre properties. These organic compounds and their transformation products are known to leach out once TRWP are deposited in the environment. This leaching may depend on a wide variety of factors, some intrinsic to the tyre and others environmental, but neither is well understood. Typically, leaching studies are done with cryogenically milled tyre tread (CMTT) particles in a solution and aliquots taken at various time points (static leaching). To increase the realism of the leaching experiments performed two key changes compared to most leaching studies are implemented: 1) Leaching is performed repeatedly with a complete solvent exchange after each step, thus better representing environmental conditions where particles are repeatedly exposed to fresh water after rain events. 2) In addition to 3 CMTT materials a TRWP material produced from the same tyres as one of the CMTT materials was tested. For 6 weeks the leachates were analysed with ultrahigh performance liquid chromatography high resolution mass spectrometry to quantify 22 tyre derived chemicals and assess many more through a suspect- and nontarget screening. With these experiments, this study aims to characterise differences between the four tyre materials to improve our understanding of how intrinsic properties influence leaching from tyres and increase the transferability from laboratory studies to the environment.

Varying leaching behaviour between different compounds is observed, with some having a large initial peak and then decreasing slowly and others showing a constant emission throughout. Sum concentrations for many compounds exceed concentrations measured in the extracts, demonstrating that even extraction may underestimate long term leaching for some chemicals. Initial results show many compounds will continue to leach with renewed introduction of water and could therefore be continuously emitted from tyre particles for a prolonged period of time. The CMTT leachates show higher concentrations of parent compounds compared to TRWP leachates where higher concentrations of transformation products were observed. This could potentially have implications for conclusions drawn on results from studies which used CMTT as a test material, which is predominantly the case.

3.21.P-Th276 Legacy of Chemical Pollution from an Underwater Tire Dump in Alver Kommune, Norway

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The long-term environmental impact of tyre pollution has been rarely studied. The site of Hjelmås, a small bay outside Bergen in Alver Kommune, provides such an opportunity. In the 1970s a producer of blast mats (which use tyres to contain explosions) went bankrupt and dumped their tyre stock into the bay. On the seabed there remains a dump of old tyres. This study investigated the legacy of this pollution source. First the area was mapped by multi-beam sonar for bathymetry and ROVs based QC Blueeye Pro revealing a 12 m x 32 m area of tyres. Sampling points were arranged along orthogonal transects from the centre of the dump and two control sites were chosen away from the tyre dump. Water samples for chemicals analysis were collected at fixed depths 1 m above the sea bottom and the top 5 cm of sediments were collected by a Van Veen grab. Metal concentrations (Cd, Co, Cr, Ni, Pb) measured by ICP-OES were negligible in the water samples, but Zn was significantly greater at some of the tyre dump sites compared to controls. In sediments, Ni, Cr, Cu, Pb and Zn were present in all samples, but greater in the tyre dump compared to control sites with Zn the major contributor to the total metal load. GC-MS/MS and LC-HRMS were employed to analyse semi-volatile and non-volatile organics, respectively. The latter method tentatively provided approximately 300 chemical entities annotated with the mzCloud database with some sites in the tyre dump showing distinction from the control sites. Phthalate acid esters were a pronounced cluster of chemicals that was further investigated. Although phthalates, particularly DEHP, were found in seawater, they were more consistently found across the sediment samples suggesting the long-term stability of the sediments for both organics and metals. In sediments, DEHP was the most prevalent phthalate, but DMP and BBzP also contributed to the sediment loads. DEHP being the most prevalent phthalate found in both environmental compartments agrees with previous findings of DEHP as the most leachable phthalate from tyre crumb rubber in seawater. Our study shows that the legacy of pollution remains in Hjelmås especially in sediments. Any efforts for remediation should ensure minimal disruption of the sediments as this may lead to increased mobilization of pollutants.

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3.21.P-Th277 Characterization of Deposited Inhalable Fraction from Recycled Rubber Flooring Degradation

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Recycled rubber flooring, widely used in playgrounds, sports tracks, and other recreational surfaces, offers a sustainable solution for managing rubber waste. However, its extensive application raises concerns about potential environmental and health risks due to the presence of different contaminants and additives like plasticizers, flame retardants, and metals. The degradation of these surfaces by various physical and chemical weathering processes results in the formation of rubber particles, some of which are small enough to be inhalable (PM_{2.5}). These remain deposited on the ground surface until they are resuspended by wind or human activity.

This study involves the characterization of potentially inhalable particles (PIP) deposited in synthetic rubber surfaces found in playgrounds and running tracks in the city of Barcelona. A total of 8 sites were sampled using a patented field resuspension chamber, that allows for the selective separation of PM_{2.5} without the issues of electrostatic charge and resuspension related to traditional methods of dust collecting. A total of 47 organic compounds were analysed, including 30 plasticizers like organophosphate esters (OPEs) and phthalates, as well as 17 flame retardants, like polybrominated diphenyl ethers (PBDEs), along with a wide range of short-chain chlorinated paraffins (SCCPs). A total of 57 metals were monitored as well.

Results vary greatly depending on the family of compounds. Plasticizers like OPEs were found at low concentrations, 18.4 ng/m² at maximum, while others like phthalates were found at up to 3039 ng/m², with compounds like diisononyl phthalate (DiNP) being the greatest contributors. Flame retardants were found at low concentrations, while SCCPs were at considerable levels, 205 ng/m² at maximum. Metals were present at great concentrations, up to $1.87 \cdot 10^5$ ng/m², with elements like Ca, Mg or Zn being the greatest contributors.

Overall, this study seeks to emphasize the presence of high quantities of concerning or regulated compounds in inhalable particles coming from parks and playgrounds. Although PIP is not directly inhalable, it can resuspend easily, as well as be absorbed by the body through ingestion and dermal contact.

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3.21.P-Th278 Human Exposure Assessment to Tire-Related Chemicals Through Urine and Silicone Wristbands: Method Development

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Tire-road wear particles (TRWPs) are generated through tire-road friction and are a significant source of non-exhaust emissions. Their accumulation in the environment raises concerns about human health due to the toxicity of Tire-Related Chemicals (TRCs), which include additives and their transformation products. Human exposure to TRCs occurs primarily through inhalation, ingestion, and dermal contact. This study aims to develop and validate methods for quantifying TRCs in urine and silicone band passive samplers. Urine, commonly used in exposome research, and silicone bands, which mimic dermal exposure, offer insights into skin exposure and internal concentrations.

Sample preparation protocols were optimized for recovery and reduced matrix effects. For silicone bands, the solvent volume, number of extractions, and reconstitution volumes were refined, with a final protocol requiring two 15-minute extractions with methanol-ethyl acetate (1:1 v/v) and reconstitution in methanol-water (1:1 v/v). Urine samples were hydrolyzed with β -glucuronidase to process metabolites, and β -glucuronidase® filtration and liquid-liquid extraction (LLE) methods were compared. While β -glucuronidase provided cleaner samples, its dilution limited detection sensitivity. LLE, with 10-fold concentration and internal calibration, allowed the detection of low-sensitivity compounds despite matrix challenges.

Silicone band extraction yielded quantitative recovery for most compounds after two extractions, validated at three concentration levels. For urine samples, LLE enabled the quantification of low-sensitivity

compounds despite matrix effects. Preliminary findings on healthy volunteers detected benzothiazoles, benzotriazoles, and cyclic amines in both matrices, with more compounds identified in urine. These validated methods provide a framework for assessing human exposure to TRCs, identifying biomarkers of TRWPs, and evaluating health risks. By comparing innovative silicone bands with traditional urine sampling, this study enhances understanding of the environmental and human health impacts of tire-related chemicals.

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3.21.P-Th279 May Saprophytic Fungi Help in the Rehabilitation of Soils Impacted by Tire Wear Particles? - A Preliminary Study

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Agricultural soils are perceived as one of the major sinks for tire wear particles (TWP). The TWP may reach soils by air, storm, or drainage water, and their deposition is potentiated by the proximity to road networks. All pathways contribute to gradual buildup of TWP in soils, with potential impacts on soil health, plant growth, and ecosystem services. Saprophytic fungi may be a promising cleansing strategy because of their natural role as decomposers. This work aimed at addressing the potential biodegradation of TWP by two saprophytic fungi (*Trametes versicolor* Tv and *Pleurotus sajor caju* Psj). For that, a 56-day experiment was conducted using a minimal medium (MSM) and a medium that simulates agricultural soil conditions (LUFA soil elutriates-ELU). The following treatments were carried out: (i) TWP in MSM or ELU with the presence of one of the fungal species; (ii) TWP in MSM or ELU without the presence of any fungal species (to allow identification of any influence of the media on its degradation); and (iii) MSM with fungi alone or ELU with fungi alone (to control for the presence of metabolites that were due to the metabolism of the fungi alone). Each treatment had four replicates with sampling points on days 0, 14, 28, 42, and 56. The biodegradation process was tracked by the production of ammonia and enzymatic activity (acid phosphatase, alkaline phosphatase and pectinase activity) in rot fungi exudates, and alterations on TWP weight loss. A higher degree of biodegradation was found in ELU than in MSM, possibly related to the presence of humic substances or the provision of a more nutrient-rich environment for fungi growth. A 7% TWP weight loss was observed in Psj+ELU on day 28. In general, the production of ammonia and the activity of the enzymes was higher in exudates of elutriates, probably for being a more nutrient-rich environment. The ammonia production and pectinase activity were higher in treatments involving the exposure of TWP to the fungi, but in those treatments, the activity of acid and alkaline phosphatase was hampered. These findings suggest the ability of fungi to tolerate possible harmful effects of the substances composing TWP and their capacity to biodegrade these particles. The primary limitations of this study are the media type and experiment duration, which may have restricted the degree of plastic breakdown. However, rot fungi have emerged as a potentially effective tactic for rehabilitating soils contaminated by TWP.

3.21.P-Th280 Ecotoxicological Impacts of Tire Wear Particles

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Anthropogenic pollution, including the rapid growth of plastic production and microplastic pollution since the 1950s, poses a severe threat to human and environmental health, with particles found globally, even in the cryosphere and human tissues. Tire wear particles (TWP) alone account for about one-third of primary microplastics in the environment, challenging biodiversity and Earth's system integrity. Therefore, supposedly environmental-friendly tire alternatives have recently appeared on the market. In this project, we compared two types of Continental tires, the conventional petro-based Grand Prix 5000 tire and the supposedly green and sustainable Taraxagum tire made from dandelion, and investigated their ecotoxicological impact. Leachates from both tire particles were used in a luminescent bacteria test (ISO

11348) to assess decomposer inhibition, a *Daphnia* Acute Toxicity Test (OECD 202) for impacts on aquatic invertebrates, a Fish Embryo Acute Toxicity (FET) Assay for developmental effects, and a MTT assay for cytotoxicity. Ready biodegradability of leachable substances was tested following the OECD 301D method.

Our results show that both Taraxagum and Grandprix TWP leachates were highly toxic to *Vibrio fischeri*, with minimal differences in IC50 values (9.1% vs. 7.6%). High toxicity was also observed for Grandprix leachates in *Daphnia magna*. FET assays indicated minimal toxicity for Taraxagum leachates, while Grandprix caused concentration-dependent developmental delays. MTT assays revealed a higher cellular toxicity for Grandprix (LC50 = 14%) versus Taraxagum (LC50 = 88%). Interestingly, biodegradation tests showed active bacterial metabolism of Grandprix leachates, but negligible degradation for Taraxagum. These data demonstrate a significant ecotoxicological risk of both TWP leachates in the environment. Grandprix TWP leachates revealed high toxicity and were biodegradable. In contrast, Taraxagum leachates were slightly less toxic, however showed negligible biodegradability, raising concerns about long-term environmental persistence. Therefore, sustainable tire materials with no ecotoxicological risk and mitigation strategies of TWPs are urgently needed.

3.21.P-Th281 Deciphering the Estrogenic Activity of Aqueous Leachates from Elastomers by Effect-Directed Analysis (EDA)

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Elastomers used in tires or membranes of hydraulic constructions contain complex mixtures of a variety of chemicals, which can be released into the environment and are potentially toxic. The aim of this study was to identify the main drivers of estrogenicity by effect-directed analysis (EDA) and to quantify their estrogenic potential. Therefore, aqueous leaching of an elastomer membrane was applied and estrogenicity was tested in a planar yeast estrogen screening (p-YES) after chromatographic separation on high-performance thin-layer chromatography (HPTLC) resulting in two active spots on the plate. For compound identification, a non-target screening approach using LC-ESI-HRMS was used. In total, 2389 features were detected in the aqueous leachate. Due to low concentrations and limited amounts, reduction of candidates by fractionation was not practicable. Thus, an exhaustive extraction of the membrane was performed using isopropanol. The organic extract was fractionated by preparative column chromatography. Fractions showing estrogenic activity were analyzed by LC-ESI+-HRMS and detected features were compared with those from the aqueous leachate. Thereby, the candidate list could be reduced to 14. Three of those were identified by library search. Authentic standards of these three substances were analyzed by p-YES and 4-hydroxydiphenylamine (4HDPA) was found to cause the same spots as observed for the aqueous leachate. 4HDPA is a transformation product of p-phenylenediamines, commonly used antiozonants in elastomers. By quantifying 4HDPA and measuring its estrogenic activity by estimating dose response relationships it was shown that about 120±30% of total estrogenicity of the aqueous leachate can be explained by 4-HDPA. In our EDA approach, it was also possible to identify 4-(phenylimino)-2,5-cyclohexadien-1-one (QMI). Our results suggested a rapid and reversible transformation of QMI into 4HDPA resulting in a complex equilibrium between these two substances and explaining the two spots in the p-YES. This together with the environmental relevance of the leaching of 4HDPA in real waters will be elucidated in follow-up studies. Moreover, further research is needed to explain further observed specific toxic effects caused by elastomers and their additives. Overall, our EDA approach is a promising tool for an efficient way to identify toxic substances released from complex materials by linking different extraction methods, HPTLC tests and chemical analysis.

3.21.P-Th282 Sorption Dynamics of Tire-Derived Compounds to Soils is a Determinant Factor for Plant Uptake

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Tires consist of diverse materials including rubber (40-50%), softeners (15%), vulcanisation agents (2-5%) and other inorganic and organic additives (2-15%). During driving, tires abrade due to mechanical and thermal stress, resulting in the emission of tire and road wear particles (TRWP). Like all plastic additives, tire-derived compounds (TDC) leach from TRWP (4-6) and may exhibit severe adverse environmental effects, for example 2-anilino-5-[(4-methylpentan-2-yl)amino]-cyclohexa-2,5-diene-1,4-dione (6PPD-Quinone), the transformation product of N-(1,3-dimethylbutyl)-N-phenyl-1,4-phenylenediamine (6PPD).

TDCs have been detected in agricultural soils and can be taken up by plants; thus, the understanding of sorption processes of TDCs to soils is important for determining the bioavailable fraction of TDCs in soils. Therefore, we investigated the sorption processes of tire-derived compounds in soil, a topic of increasing concern due to the potential environmental and health impacts.

We investigated the sorption kinetics of selected TDCs, including aniline, 6PPD-Quinone, hexa(methoxymethyl)melamine (HMMM), 1,3-diphenyl guanidine (DPG), 5-methyl-1h-benzotriazole (5-Me-1H-BTR) and benzothiazole (BTH) using two reference soils with distinct organic carbon contents and cation exchange capacities. Sorption isotherms were generated for four well-characterised reference soils along with two soils from Israel. The experiments followed OECD guideline 106 for batch sorption experiments. TDC concentrations were quantified using triple quadrupole liquid chromatography-mass spectrometry. To elucidate the soil properties influencing TDC sorption, soil mineral composition was characterised through X-ray diffraction analysis.

The sorption kinetics revealed that all compounds followed pseudo-second-order kinetics, indicating that TDC sorption in soil involves dynamic processes, consistent with literature on the sorption of organic compounds in soils. In this model, chemisorption is the dominant mechanism, suggesting the involvement of intermolecular interactions such as covalent bonding, π - π interactions, or cation exchange. The sorption behaviour of TDCs in soils is crucial for determining their bioavailability, which can be assessed through distribution coefficients that allow to estimate the potential risk of plant uptake.

3.21.P-Th283 Monitoring of Tire-Derived Compounds in Commercial Leafy Vegetables and Associated Dietary Exposure

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The toxicity of tire particles has been linked to leachable organic additives and associated transformation products (tire-derived compounds). Tire particles, and tire-derived compounds are introduced to the agricultural environment via atmospheric deposition, irrigation with treated wastewater, and biosolids (treated sewage sludge) application as fertilizer. In the agricultural environment, tire-derived compounds may be taken up by edible plants, leading to human exposure. Sixteen tire-derived compounds were measured in twenty-eight commercial leafy vegetable samples grown in Switzerland, France, Spain, and Israel. In commercial leafy vegetables, six tire-derived compounds were detected: benzothiazole (maximum concentration 238 ng/g dry weight), 2-hydroxybenzothiazole (maximum concentration 665 ng/g dry weight), 1,3-diphenylguanidine (maximum concentration 2.1 ng/g dry weight), N-(1,3-dimethylbutyl)-N²-phenyl-p-phenylenediamine (6PPD, maximum concentration 0.4 ng/g dry weight), N-Isopropyl-N-phenyl-4-phenylenediamine (IPPD, maximum concentration 0.1 ng/g dry weight), and N-phenyl-N-cyclohexyl-p-phenylenediamine (CPPD, maximum concentration 0.3 ng/g dry weight). At least one compound was present in 71% of samples analyzed. Statistical analyses did not reveal correlation between known growth conditions and tire-derived compound concentrations in the leafy vegetables. Based on measured concentrations and dietary survey data, the estimated daily intake due to leafy vegetable consumption of these tire-derived compounds under a mean and maximum concentration scenario was calculated. The estimated daily intake for 1,3-diphenylguanidine ranged from 0.05 ng/person/day in the mean scenario to 4.0 ng/person/day in the maximum scenario; benzothiazole ranged from 12 to 1,296 ng/person/day; 6PPD ranged from 0.06 to 2.6 ng/person/day; IPPD ranged from 0.04 to 1.1 ng/person/day; CPPD ranged from 0.05 to 2.6 ng/person/day. The estimated daily intake via leafy vegetable consumption was generally lower than or comparable to exposure via other known sources, although leafy vegetables represent only a fraction of total dietary exposure. Future studies should elucidate pathways of tire-derived compounds from road to field, assess the exposure to transformation products, and investigate the biological effects associated with this exposure.

3.21.P-Th284 Toxicity of Plastic Additives to *Daphnia magna* and *Lemna minor* and the Temporal Trends of their Leaching from Microplastics

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Microplastics (MPs) are a complex group of environmental contaminants with varying physical and chemical properties. They consist not only of the polymer chains but also of many different kinds of

additives that are used to modify the properties of plastic mass. The additives are not chemically bonded to the polymer chains and can leach out of the plastic mass over time, thus causing potential harm to the organisms in the surrounding environment. Still, the majority of MP-related research focuses on physical particles rather than the released additives.

In this study, we wanted to assess the toxicity of different plastic leachates to *Daphnia magna* and *Lemna minor* in the absence of physical MPs. Both *D. magna* and *L. minor* are common freshwater organisms and vital parts of the aquatic food web. In addition, we wanted to evaluate temporal trends of additive leaching and elucidate the molecular identity of additives released.

Leachates from nine different MPs were prepared in environmentally relevant concentrations (10 g/l) over three subsequent leaching cycles (20d, 160 rpm, ambient temperature, darkness). After each leaching, the MPs were removed via filtration (0.22 µm pore size membrane). Then, the toxicity of obtained leachates were tested on *D. magna* neonates (<24h old) in different concentrations (5, 20, 50 and 75%) by monitoring their mobility at 24 and 48h according to the OECD (2004) guidelines as well as at 72 and 96h. Leachate toxicity data will be complemented with results from *L. minor* growth inhibition tests (OECD, 2006). Identification of the leached additives using untargeted high resolution LC MS is currently an ongoing endeavor and will later be complemented with ICP MS analyses for elemental characterization of the leachates.

Our current results indicate that tire rubber leachates can induce acute mortality on *D. magna* already at 20% concentration, thus making the determination of any EC_{xx} values impossible. The other leachates did not show acute toxicity to *D. magna* within the 96h test period, but their toxicity cannot be ruled out before the tests with *L. minor*. Ideally, the high resolution LC MS analyses will provide us with the identity of released additives and single out possible candidate molecules responsible for the toxicity observed thus far.

Overall, our study demonstrates the important role of plastic additives in ecotoxicology and the hazards they may pose to the environment already at relatively low concentrations.

3.21.P-Th285 Toxicity of Tyre Wear Particle Leachates to Lichens

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Tyre wear particles (TWP) are some of the dominant sources of microplastics in the aquatic and terrestrial environment. Once TWP enter ecosystems, they can leach certain plastic additives that can be potentially toxic to biota. However, little is known about the impact of TWP on non-target organisms. This study aims to determine the acute toxic effect of leachates derived from different TWP on two lichen species: the sensitive *Ramalina* and resistant to pollution *Xanthoria parietina*, using different physiological and biochemical endpoints. Leachates were obtained by incubating 1 g L⁻¹ of TWP in water. Each lichen species was exposed to leachates at five different concentrations for 1 h, and endpoints were measured every 24 h over 3 days. Leachates from TWP were toxic to lichens. Our results suggest that TWP leachates adversely affect lichen viability, although more field data on TWP and their leachate concentrations are needed to fully assess the environmental impact of TWP.

3.21.P-Th286 Toxic Effects of Tire Wear Particle Leachate on Survival, Detoxification, Antioxidant System, and Energy Metabolism of Two Marine Zooplankton

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Tyre wear particles (TWPs) are particulate pollutants generated by friction between tire wear and road surfaces. TWPs on roadways can be transported to the ocean via wind or surface runoff and thus are recognized as an emerging contaminant in marine surface water. In the ocean, harmful chemicals associated with TWPs, such as heavy metals, polyaromatic hydrocarbons (PAHs), and phenolic compounds, can leach into water fraction, which can induce toxic effects on marine biota. However, toxic effects of chemicals associated with TWPs on marine organisms are not well-understood. In this study, we obtained TWP leachates (5 gTWPs/L for 3 days) from two commercially available tires (one for truck and one for sedan) and investigated toxic effects of these TWP leachates and zinc (Zn), typical pollutants in tires, on survival, reproduction, transcriptional modulation and energy reserves of two marine zooplankton, the brackish water flea *Diaphanosoma celebensis* and the marine rotifer *Brachionus koreanus*. Zinc (Zn) was identified as the dominant chemical in both TWP leachates (1.96 and 5.77 mg/L in truck and sedan leachates, respectively). Acute toxicity test showed that Zn is highly toxic to both

species (48-h LC50Zn²⁺: 2.13 and 2.47 mg/L for *D. celebensis* and *B. koreanus*, respectively). And both truck and sedan TWP leachates were also highly toxic to *D. celebensis* (48-h LC50: 49.31 and 24.95%, respectively, equivalent to 0.96 and 1.44 mgZn²⁺/L, respectively). Similarly, truck TWP leachate induced significant mortality to *B. koreanus* (48-h LC50: 65.82%, equivalent to 1.29 mgZn²⁺/L), but sedan TWP leachate induced less than half of mortality of *B. koreanus*, even at the 100% concentration which contain Zn more than median lethal concentration, suggesting antagonistic toxicity of Zn. Chronic toxicity test showed reproductive disorder of both species exposed to Zn and TWP leachates, and gene expression, enzyme activity, and cellular molecules related to detoxification, antioxidant system, and energy metabolism were significantly modulated following exposure to Zn and both TWP leachates. Our findings imply that TWP leachates can disrupt reproduction, antioxidant system, and energy metabolism of marine zooplankton and suggest that Zn is one of the major sources determining toxicity of TWPs but they can toxicologically interact with other chemicals in TWPs.

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3.21.P-Th287 Adverse Effects of Tyre Particles on Estuarine Invertebrates and Microbial Communities

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Tyre particles emanating from vehicles can be readily transported into the natural environment. Approximately 2% of tyre particles entering the environment are predicted to end up in estuaries, with mean environmental concentrations of tyre particles ranging 0.5-2 g kg⁻¹ and highest observed concentrations >25 g kg⁻¹. The toxicological and ecological risks posed by tyre particles remain poorly understood for a diverse range of taxonomic groups, with a paucity of chronic exposure studies using environmentally relevant parameters. To elucidate the ecological risks tyre particles pose to estuarine ecosystems, a chronic dose-response exposure study was conducted. Natural estuarine sediments were spiked with 0-100 g kg⁻¹ of tyre particles derived from end-of-life passenger car tyres. Lugworms (*Arenicola marina*) and cockles (*Cerastoderma edule*) were exposed to 1 kg of sediment, with 1350 mL overlying seawater, for 28 days. Mortality, feeding and burial activity were monitored throughout, and gene expression, burial time, growth and shifts in sediment microbial communities were assessed post-exposure. Tyre particles caused lethal toxicity in lugworms, with 100% mortality at concentrations >25 g kg⁻¹, and a LC5028d of 11.9 g kg⁻¹. Tyre particle concentrations >1 g kg⁻¹ caused significant decreases in lugworm feeding, burial and weight, and the burial behaviour of cockles. Exposure to tyre particles caused significant shifts in sediment microbial community composition and diversity, for both prokaryotic and eukaryotic organisms. The study highlights that environmentally relevant concentrations of tyre particles can have substantial ecological effects in estuarine ecosystems.

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3.21.P-Th288 Assessment of the Toxicity of Tire Wear Particles and Their Leachates Using a Cell-Based Toolbox from Rainbow Trout (*Oncorhynchus mykiss*)

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6PPD-quinone (6PPD-Q), formed by the environmental oxidation of the tire wear antioxidant 6PPD, has been identified as one the causes of urban runoff mortality syndrome (URMS), responsible for the mass death of coho salmon on North America's western coast. Understanding the impact of Tire Wear Particles

(TWPs) and their chemicals, including 6PPD, on aquatic species is critical to assessing risks to the ecosystem. Cryogenically milled tire tread (CMTT) can be used as suitable surrogates to experimentally study the toxicity of TWPs and their associated chemicals with in vitro tests, which are innovative, fast, and efficient tools that offer a less time- and resource-consuming alternative to traditional in vivo testing. We developed a cell-based toolbox from rainbow trout (*Oncorhynchus mykiss*) to assess in vitro the potential harmful effects on fish. These cells, named RTgill-W1, RTgutGC and RTbrain, derived from the gill, intestine, and brain tissues respectively, can provide important insights into the mechanism of action of and tissue-specificity of these pollutants. This method, initially developed for the RTgill-W1 cell line and validated in the corresponding OECD Test Guideline N° 249, was extended to the other cell lines. With these cells, we studied acute toxicity of particles and chemicals by assessing their effects on several biological endpoints: cell metabolic activity, lysosome, and cell membrane integrity. Different types of CMTT, representative of the European and US markets, were tested on RTgill-W1 cells, revealing similar toxic effects on the three endpoints investigated. Interestingly, the 6PPD-Q, present in both types of CMTTs, was found to be particularly toxic to RTbrain but not to the other cell lines, confirming the suitability of these cell lines for determining tissue-specific modes of action. Our next steps involve: (i) examining molecular toxicity mechanisms through transcriptomics and proteomics; (ii) expanding toxicity endpoints of CMTT and their associated chemicals; (iii) evaluate chronic exposure to CMTT and iv) study the trophic transfer effects by testing invertebrates feed with biofilm contaminated by CMTTs. These cell-based in vitro assays hold potential to advance understanding on the toxicological risks of tire wear particles and their tire-associated chemicals to aquatic organisms while considerably reducing animal testing.

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3.21.P-Th289 Integrating Transcriptomic Points of Departure (tPODs) with Bio- and Chemical Analyses for Hazard Assessment of Road Runoff in Zebrafish

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Road runoff and tire wear particles can severely impact freshwater ecosystems, such as the Urban Runoff Mortality Syndrome in coho salmon (*Oncorhynchus kisutch*) along the northern Washington coast, USA. However, little is known about the environmental impacts of urban runoff in other regions, particularly Europe. The RoadTox project aims to address this gap by integrating bioanalytical, chemical, and molecular methods to study the effects of road runoff on zebrafish larvae. Specifically, three consecutive stormwater samples were collected from the BAB4 motorway in Aachen, Germany, and zebrafish embryos were then exposed to graded concentrations of total and particle-free runoff fractions. Our analyses included acute toxicity, enzymatic biomarkers, neurotoxic endpoints, and whole transcriptome analysis. Transcriptomic Points of Departure (tPODs) were then calculated based on benchmark concentrations of individual genes for which concentration-response models could be fitted. Acute toxicity varied highly among the three samples, with EC10 thresholds ranging from 2.3% to 28% runoff for total samples and 9.8% to 45% for filtered samples, confirming that particulate matter contributed most to toxicity. Additional effects were observed on spontaneous tail coiling, light-dark transition behaviour, and EROD activity differing in severity between samples and fractions. Transcriptional analysis revealed an increasing number of transcripts that were dysregulated with increasing concentrations of all three runoff samples. Preliminary tPOD results showed effect thresholds ranging from one-quarter of the EC10 concentration to nearly the EC10, within the single-digit percentage range of road runoff. Noteworthy, upregulation of *cyp1A*, linked to dioxin-like activity and oxidative stress, was consistently observed across all samples, matching previous observations of cardiovascular teratogenic effects and enzyme biomarker data. Other dysregulated genes (e.g., *cyb5a*, *alox5b.2*, *cbr11*, *sult6b1*) were associated with

oxidative stress and xenobiotic metabolism, which may be related to exposure to PAHs and benzothiazoles as likely drivers of toxicity. The results of this study, funded by the Ministry for Environment, Agriculture, Conservation, and Consumer Protection of North Rhine-Westphalia (MULNV), Germany, demonstrate that road runoff affects gene expression at low concentrations and provide a foundation for identifying toxicity drivers through molecular pathway analyses.

3.21.P-Th290 CMTT Exposed Periphyton Induces Bottom-up Food Chain Effects in the Freshwater Grazer *Physa acuta*

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Periphyton communities grow on the bottom of freshwater streams, being composed of a complex mixture of microorganisms embedded in extracellular polymeric substances (EPS). These microbial communities form the base of aquatic food webs being crucial to ecosystem functioning. Consequently, changes at this level can have far-reaching effects on onward food-web relationships. They also may act as a sink for pollutants, including particles, such as tire and road wear particles (TRWP), which have been detected in streams and rivers not exceeding 10 mg/L. We have previously shown that CMTT (cryogenically milled tire tread) particles, used as proxies for TRWP, accumulate in periphytic communities though with little structural or functional impacts for these communities at environmental relevant concentrations. However, the potential threat to organisms grazing on CMTT-contaminated periphyton has not yet been explored. We hypothesized that CMTT particles associated with the periphyton matrix, will be transferred to *Physa acuta*, and have potential to interfere with the snail's health. To explore this hypothesis, we fed snails on CMTT contaminated periphyton: near natural periphyton was grown until mature (14d) and subsequently exposed to nominal concentrations of 50 mg/L of CMTT particles, leachates obtained from 50 mg/L CMTT and 2 µg/L of 6PPD-Q (compound highly toxic for fish) for 96h. The exposed periphyton was offered to snails in contamination-free microcosms for 14d, with medium and food renewal at 7d. After the exposure period, snails were transferred to new microcosms for a 24h depuration period. The snail's health was evaluated via feeding rate, faeces production, growth, and reproduction. About 20% mortality was observed for the leachates treatment, while particles only and 6PPD-Q did not induce statistically significant changes in mortality compared to the control. Leachates and CMTT treatments induced higher feeding rates and growth compared to control. The 6PPD-Q exposed snails tended to remain attached to the glass when not feeding, had lower feeding rates and less eggs per clutch compared to the other treatments. Moreover, snails only laid egg clutches on the biofilm in the control, while for the other treatments the egg clutches were attached to the microcosm glass. These visual observations will be analysed further in upcoming behavioral experiments. Analyses on the transfer and accumulation of particles and leachates into the snails are ongoing

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3.21.P-Th291 Evaluating the Effects of 6PPD-Quinone Exposure on Early-Life Stage Rainbow Trout Transcriptome

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The rubber tire derivative N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone), commonly found in roadway runoff, has recently been identified as a key driver of urban runoff mortality syndrome in coho salmon. Subsequent studies have shown significant variability in species sensitivity to this toxicant, even within the salmonid family. While traditional exposure studies are essential for assessing species sensitivity, transcriptomic analysis offers a broader perspective, uncovering gene expression changes and disruptions in biological pathways, as well as establishing transcriptomic points of departure (tPODs). To investigate early-life stage toxicity, a test was conducted on rainbow trout (*Oncorhynchus mykiss*) alevins starting at hatch. Treatment groups included a solvent control (0.01%

dimethyl sulfoxide), and time-weighted average 6PPD-quinone concentrations of 0.06, 0.10, 0.20, 0.44, 1.30, and 2.35 µg/L. After 96 hours of exposure, whole-body alevins were sampled and flash-frozen for transcriptomic analysis. The results revealed over 1,200 disrupted genes at a log₂ fold change of 2.0, with significant alterations in biological pathways, including osteoclast differentiation, apoptosis, necroptosis, inflammation, and immune response. Evaluation of tPODs found similarity between tPODs and sub-chronic mortality data for the same species and life stage, indicating that tPODs may be used predictive of sub-chronic mortality.

3.21.P-Th292 Transcriptomic Disruption of Northern Leopard Frog Tadpoles (*Lithobates pipiens*) by 6PPD-Quinone

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The rubber-tire derivative N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-q) has recently been identified as a potential cause of Urban Runoff Mortality Syndrome, which describes the widespread deaths of pre-spawn salmon near roadways. Tire-wear particles deposited on road surfaces are washed into runoff during rainfall, introducing pulses of 6PPD-q into aquatic environments. Fish species have shown widely varying sensitivity to this toxicant, and environmental concentrations often exceed sensitivity thresholds for certain species, posing a significant risk to vulnerable aquatic organisms. However, the potential toxicity of 6PPD-q to amphibians remains unexplored.

Larval-stage amphibians, such as northern leopard frog (*Lithobates pipiens*; NLF) tadpoles, frequently inhabit small, temporary water bodies near roadways, where they are exposed to roadway contaminants like copper, which is known to harm amphibian populations. To investigate, an acute 96-hour exposure was conducted with freshly hatched NLF tadpoles. Treatments included nominal 6PPD-q concentrations of 20, 2, and 0.2 µg/L, along with a 0.01% dimethyl sulfoxide control, with three replicates per treatment. At the conclusion of the exposure, tadpoles were flash-frozen, and total RNA was extracted from three pooled tadpoles per replicate. Transcriptomic analysis was performed using the EcoToxChip NLF v0.1 and analyzed with EcoToxExplorer.

Although no mortality occurred during the exposure, transcriptomic disruption was observed. At a log₂ fold change of 0.5 and a p-value <0.05, nine genes were significantly dysregulated, with eight downregulated and one upregulated. These findings suggest a potential risk of sub-lethal effects to NLF tadpoles exposed to 6PPD-q. Further research is needed to better understand exposure pathways and assess the broader risks of 6PPD-q to amphibian populations.

3.21.P-Th293 Toxicokinetics and Biotransformation Products of 6PPD-Quinone Using In Vitro Substrate Depletion Assays in Salmonids

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N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) is an oxidation product of rubber tires that is acutely toxic to select salmonid species at environmentally relevant concentrations created by stormwater runoff events. 6PPD-quinone exhibits marked species-specific sensitivity, with closely related salmonids ranging in acute toxicity thresholds by several orders of magnitude. Recent studies investigating the mechanism of action of 6PPD-quinone have suggested that differences in biotransformation rate and metabolic pathways may explain some of the stark differences in lethality observed across a relatively well conserved phylogenetic space. The present study explores the toxicokinetics of 6PPD-quinone in rainbow trout (*Oncorhynchus mykiss*) and arctic char (*Salvelinus alpinus*) to represent a sensitive and insensitive species respectively, using primary cells from liver and

gill tissue. Primary hepatocyte suspensions were isolated using a standardized guidance approach (Organisation for Economic Co-operation and Development) OECD Test Guideline 319A) to measure intrinsic clearance of 6PPD-quinone, as well as appearance of transformation products. Primary gill cells were isolated using a previously published protocol, and biotransformation was assessed with a modified 319A approach. Samples were analyzed using liquid chromatography high-resolution mass-spectrometry (LC-HRMS) to quantify parent compound depletion as well as metabolite formation. We hypothesize that differences in intrinsic clearance of 6PPD-quinone between these two species within liver and gill tissues will be predictive of overall acute toxicity differences. The quantification of 6PPD-quinone transformation products further serves to investigate these differences. This study demonstrates the adaptability of substrate depletion based primary cell assay methods by applying them to an emergent, acutely lethal contaminant with a complex toxicity outcome.

3.21.P-Th294 Assessing Epithelial Membrane Integrity and Permeability in Rainbow Trout RTgill-W1 and Atlantic Salmon ASG-10 Gill Cells Exposed to 6PPD-Quinone

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The compound N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) has been linked to significant mass mortalities of coho salmon along the western coast of North America. This toxicant forms from the antioxidant 6PPD through the environmental oxidation of tire wear particles, which enter aquatic systems via urban stormwater runoff. Although the acute lethality of 6PPD-quinone at concentrations $\geq 1 \mu\text{g/L}$ has been established in various salmonid species, sensitivity to this toxicant varies greatly among fishes. The mechanism behind its species-specific acute toxicity remains unknown.

Evidence suggests that 6PPD-quinone may cause molecular dysregulation of genomic pathways related to cell adhesion and endothelial permeability. Therefore, this study aims to evaluate the impact of 6PPD-quinone on the integrity and permeability of a modeled gill epithelium using two salmonid cell lines: RTgill-W1 (rainbow trout, sensitive species) and ASG-10 (Atlantic salmon, tolerant species). Three key targets of cell viability will be assessed: lysosomal integrity using the neutral red assay, metabolic activity using the MTT assay, and cell membrane integrity using the LDH assay. Additionally, gill cells will be cultured on a semipermeable insert that separates apical and basolateral compartments. Once they reach confluence, cells will be exposed to incremental concentrations of 6PPD-quinone for specified durations in 24-well plates. Transepithelial electrical resistance (TEER, in ohms) will be measured with chopstick electrodes to quantify the barrier integrity. Additionally, paracellular flux will be measured using FITC-dextran as well as the use of targeted qPCR to assess changes in abundance of transcripts of genes coding for specific cell junction proteins. Given that the gill epithelium is a primary target site for water-borne pollutants, this research aims to reveal the effects of 6PPD-quinone on gill permeability in both sensitive and tolerant species as a potential mechanism of toxicity.

3.21.P-Th295 A broad approach to understand how 6PPD-q, combined with phenanthrene, affects thermal tolerance, hematology, and omics in three salmonid species

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Because of climate change, urbanization, and hydrological manipulation of water flows, water temperatures in urban environments are increasing, which has implications for freshwater fish that inhabit cooler streams and rivers. An additional stressor that fish in urban settings must cope with is stormwater, a complex chemical mixture, including many chemicals that originate from car tires, including antioxidants and their transformation products, such as 6PPD and 6PPD-quinone, and polycyclic aromatic hydrocarbons (PAHs), such as phenanthrene. Recently, 6PPD-quinone was identified as the cause of urban-runoff mortality syndrome of Coho salmon (*Oncorhynchus kisutch*) in the Pacific Northwest. Since, the discovery of 6PPD-q, environmental and toxicological understanding of 6PPD-q has progressed fast and 6PPD-q is now detected ubiquitously in urban aquatic systems, while the mechanism of acute lethality has been linked to disruption of mitochondrial functions. However, we know very little about how other contaminants in stormwater runoff influence the toxicity of 6PPD-quinone. In addition, the effects of 6PPD-q alone, and in combination with other stormwater related chemicals, on thermal tolerance in fish is not known. To fill this knowledge gap, we investigated how exposure to a road-runoff surrogate-mixture of 6PPD-quinone ($0.5 \mu\text{g l}^{-1}$) and phenanthrene ($5 \mu\text{g l}^{-1}$) influenced the thermal avoidance behaviour of

three salmonid species: brown (*Salmo trutta*), bull (*Salvelinus confluentus*), and rainbow trout (*Oncorhynchus mykiss*). Additionally, we investigated body burden, xenometabolome, gill and liver transcriptomes, and plasma-related parameters. Bull trout exposed to the individual components and the mixture presented thermal avoidance behaviour at significantly lower temperatures (mean range: 17.7 to 19.1°C) compared to control (20.7 ± 0.4°C). No such difference was observed among brown trout. Interestingly, a 5.7 and 7.3 percentage unit reduction in hematocrit was observed among 6PPD-q and mixture exposed brown trout, respectively. No reduction in hematocrit were observed among bull trout, although their blood was observed to be more viscous than control, suggesting altered hematology. Assays with rainbow trout are in progress. Comparative and species-specific molecular processes and biomarkers are to be investigated.

3.21.P-Th296 Assessing Ecological Impacts of 6PPD-Q in Aquatic Ecosystem Using Mesocosm: Focusing on the Fish Toxicity

Yooeun Chae¹, **Hyungmin Kim²**, **Jung Eun Min¹**, **Wonman Kang¹** and **Sooyeon Kim¹**, (1)Korea Institute of Toxicology (KIT), "Korea, Republic of", (2)Korea Institute of Toxicology (KIT), "Korea, Republic of" 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine), an additive used as an antioxidant and anti-degradation agent for tires, has received worldwide attention for its extraordinary toxicity. In particular, the oxidized product 6PPD-Q, which is produced by the reaction of 6PPD with ozone, is highly toxic to salmon, so it is urgent to study the effects of these substances on aquatic ecosystems. Therefore, this study aimed to evaluate the chronic effects of 6PPD-Q on native fish species living in Korea by creating a simulated ecosystem that mimics the actual water environment, and to determine the environmental behavior and biological effects of the substance. The experiment was conducted for 60 days, and the half-life of 6PPD-Q in water bodies under the experimental conditions was found to be less than 24 hours. The concentration of 6PPD-Q in aquatic plants was not found to increase significantly with exposure. This suggests that 6PPD-Q is rapidly degraded or translocated in water and does not accumulate in plants. The plankton community in the water body of the simulated ecosystem was sampled periodically during the exposure period to evaluate the effects of 6PPD-Q on plankton community characteristics, and it was found that daphnia, caddisflies, water snails, allegorical insect larvae, and invertebrate communities were formed. In addition, an omics-based chronic toxicity assessment based on different pathways was performed using carp, and it was found that 6PPD-Q had some effects on fish. This study allowed the evaluation of the effects of 6PPD-Q, an oxidized product of tire additives, on the aquatic environment, and is expected to help predict the ecological effects of long-term and continuous environmental exposure to these substances in advance, and to respond to substance use regulation and ecosystem protection.

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3.21.P-Th297 Sperm Genotoxicity of the Rubber Tire Oxidant By-Product, 6PPD-Quinone

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N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-q) is a transformation product of the tire rubber antioxidant 6PPD. 6PPD-q reaches the aquatic environment by stormwater and urban runoff and was associated with high mortality of salmon. Several studies evaluating acute toxicity were performed in different aquatic species showing a wide range of toxicity values. Although 6PPD-q is known to cause oxidative stress and mutagenicity to bacteria, there is limited information about other sublethal effects, especially in invertebrates. We had previously observed that 6PPD-q was not toxic to the marine amphipod *Parhyale hawaiiensis*, but induced micronuclei in hemocytes after 96 h exposure. The aim of this study was to complement the genotoxicity evaluation of 6PPD-q with the marine amphipod *P. hawaiiensis* using the comet assay in sperm cells. 6PPD-q was dissolved in DMSO at the limit of solubility and tests were performed using a maximum of DMSO of 0.01%. *Parhyale hawaiiensis* male adults (<8 months old) were exposed to 50 and 500 µg/L 6PPD-q for 96 h then sperm cells were collected. DMSO was used as solvent control and ethyl methanesulfonate 2mM was used as positive control. Sperm cells from 16 organisms per condition were used for comet assay and from 2 organisms per condition to assess sperm viability using Live/Dead Sperm Viability Kit (with SYBR-14 and propidium iodide). Sperm viability was above 90% for the two concentrations tested. DNA damage in sperm cells increased after exposure to both concentrations of 6PPD-q compared to the solvent control. We conclude that 6PPD-q was genotoxic to sperm cells of *P. hawaiiensis* therefore has the potential to cause long term effects

especially at population levels. Our next step will be to evaluate possible effects of 6PPD-q in the embryo development after parental exposure of males and females to this compound.

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3.21.P-Th298 Assessment of Adsorption and Leaching Potential of 3H-6PPD Quinone in Soils

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This study was conducted to evaluate the adsorption and leaching behavior of 3H-6PPD-quinone in three soil types with varying pH levels, clay contents, and organic matter.

The adsorption tests were performed using the indirect method with a soil-to-aqueous phase ratio of 1:5 (soil/0.01 M CaCl₂). Adsorption equilibrium was achieved within 7 hours for all three soils. After 48 hours, the adsorption coefficients (K_d) were determined to be 70.8 cm³/g for Loam soil, 43.3 cm³/g for Silt Loam soil, and 20.4 cm³/g for Sandy Loam soil. The organic carbon-normalized adsorption coefficients (K_{oc}) calculated after 48 hours were 1727.6 cm³/g for Loam soil, 1731.0 cm³/g for Silt Loam soil, and 3350.0 cm³/g for Sandy Loam soil. Based on the K_{oc} values, which are indicative of mobility in soil, 6PPD-quinone was classified as having Low Mobility in Loam and Silt Loam soils and Slight Mobility in Sandy Loam soil.

Leaching tests were conducted using atrazine (Relative Mobility Factor [RMF]: 1.03) as a reference compound. For the analysis of 3H-6PPD-quinone, soil samples were extracted with various organic solvents. The percentages of the test compound detected in the 0-6 cm soil sections were 95.0% for Loam soil, 94.2% for Silt Loam soil, and 92.3% for Sandy Loam soil. Compared to atrazine, the RMF values for 3H-6PPD-quinone were 0.5 in Loam soil, 0.3 in Silt Loam soil, and 0.2 in Sandy Loam soil. Based on these RMF values, 6PPD-quinone was classified as Slightly Mobile in all three soil types.

3.21.P-Th299 Permeability and Toxicity of Tire-Derived PPDs and Their Transformation Products in Human Gut Cells

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Tires contain thousands of chemicals, many of which can become environmental pollutants upon release. Among these, N,N'-disubstituted phenylenediamines (PPDs) are commonly added to tires as anti-aging agents, protecting against oxidative degradation to maintain tire performance. However, PPDs are highly reactive with ozone and oxygen, forming transformation products (TPs) such as PPD-quinones, which can accumulate in tire and road wear particles and disperse through environmental compartments via sorption, leaching, and runoff. One widely used PPD, N-phenyl-N'-(1,3-dimethylbutyl)-p-phenylenediamine (6PPD), along with its toxic transformation product 6PPD-quinone (6PPDQ), has been linked to acute mortality syndrome in coho salmon, raising urgent concerns about adverse effects on human health. Human exposure to PPDs and their TPs occurs through ingestion, inhalation, and contaminated water, and these substances have been detected in human urine, breast milk, and blood. This study addresses the need for a comprehensive health risk assessment by investigating the cellular permeability and cytotoxicity of seven PPDs (IPPD, CPPD, DPPD, 6PPD, 7PPD, 8PPD, and 77PD) and four TPs (IPPD-Q, CPPD-Q, DPPD-Q, and 6PPD-Q) in human intestinal (Caco-2) epithelial cell line. Mitochondrial activity, membrane integrity and oxidative stress were assessed using WST-1, LDH and ROS assays, respectively, at realistic (non-lethal) concentrations. Both individual and cocktail effects were scrutinized to determine the mode of interaction, whether additive, synergistic, or antagonistic. Chemical concentrations were quantified at the beginning and end of exposure using liquid-liquid extraction with acetonitrile, followed by LC-MS/MS analysis. Structure-activity relationships were evaluated to identify structural features linked to toxicity, with a focus on the role of hydrophobicity. The findings provide critical insights into the effect of these chemicals on human cells, stressing the need for their inclusion in environmental monitoring programs to protect public health by tracking exposure levels and informing regulatory actions.

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3.22 Matching Microplastic and Nanoplastic Reference Materials to Exposure Scenarios

3.22.T-01 Nano-Sized Polypropylene as a Promising Candidate Reference Material: Preparation, Characterization and Stability in Complex Matrices

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The lack of nanoplastic (NPs) reference materials capable of mimicking real-world scenarios is currently hampering the development of validated extraction techniques from complex matrices (e.g. soils, sediments, biota), the development of analytical approaches for robust identification and quantification, and the ability to use environmentally relevant test materials in (eco)toxicity testing and hazard assessment.

Here, we present a strategy towards the development of a potential NP reference material based on nano-sized polypropylene particles (nanoPP) developed within the EURAMET-funded PlasticTrace project (<https://plastictrace.eu/>). The strategy includes the preparation, the physicochemical characterization and the stability assessment of nanoPP in suspension both in its pristine state, but also when spiked into food (mineral water, milk) and environmental matrices (river water).

NanoPP was produced reproducibly in a top-down approach by crushing in acetone with an UltraTurrax, filtering and change of solvent to MilliQ water. The prepared nanoPP material was subjected to a comprehensive physicochemical characterization including e.g., dynamic light scattering (DLS), particle tracking analysis (PTA) and asymmetrical-flow field-flow fractionation coupled with multi-angle light scattering (AF4-MALS) for particle size distribution assessment, PTA and scanning electron microscopy (SEM) for particle number concentration determination, SEM and atomic force microscopy (AFM) for shape analysis and pyrolysis GC-MS for chemical identification. Performed studies showed that the prepared nanoPP material is irregularly shaped and highly polydisperse with a particle size of around 180 nm (e.g., Dh-z-ave from DLS) and a particle size distribution from around 15 nm up to 135 nm (radius of gyration Rg from AF4-MALS).

Storage stability studies using DLS, PTA and AF4-MALS revealed nanoPP in aqueous suspension to be stable for at least 22 months with respect to particle size distribution and number concentration rendering it a promising NP candidate reference material. However, conducted spiking experiments in mineral water, river water and milk showed a time-dependent agglomeration of nanoPP that needs further investigation with focus on dedicated sample preparation and extraction techniques, as well as enrichment protocols from complex food and environmental matrices.

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3.22.T-02 Advancing Environmental Fate Assessments: Generation and Tracking the Fate of Realistic (UV-Degraded) Nanoplastics

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Reference materials constitute an important part for nanoplastic fate studies: they ensure comparability of results between labs, are used for calibration of analytical techniques, facilitate method development and can be used as benchmarks for toxicological assessments. However, reference materials for microplastics are only rarely available and, moreover, are not representative of environmental nanoplastics.

To understand the environmental fate of nanoplastics we tested two approaches to obtain more realistic nanoplastic materials (including extensive physicochemical characterization):

1) solvent precipitation using polyamide-6 (PA-6) resulted in non-spherical fragmented particles with a particle size distribution under 500 nm

2) Ultraviolet (UV) aging of an acrylic paint followed by a re-dispersion step enabled the sampling of more realistic multi-component UV-aged micro- and nanoplastic fragments < 40 µm

The generated fragments were used for the development of methods to extract micro- and nanoplastics from compost. The extraction protocols are based on deagglomeration and size fractionation to isolate the

nanoplastics from compost components, which are then enriched using a stacked density-separation technique. To demonstrate the identification of individual micro- and nanoplastic fragments, we performed spiking tests and measurements via infrared nanoimaging using Scattering-type Scanning Near-field Optical Microscopy (s-SNOM), as well as Raman and fluorescence microscopy. The final goal is the identification and quantification of individual realistic fragments in environmental matrices. However, each material, extraction protocol and analytical technique comes along with specific challenges that need to be addressed and improved. This can only be achieved when suitable reference materials are available.

3.22.T-03 Harmonisation of Sample Preparation for the Microplastic Analysis: Recovery Rates of Tablets Containing Microplastics as Reference Material for Food and Environmental Matrix

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Microplastics (MP) are everywhere and their impact on health and environment is still discussed, based on the precautionary principle, their presence should be monitored and emission should be reduced. The crucial point is the reduction of MP pollution but also the introduction of legally binding, standardized procedures for their analysis. The limited availability of certified reference material as well as absence of standard operating procedures to identify, characterize and quantify MP according to number and mass have generated lack of comparability between analytical methods and laboratories. First, we developed tablets of a water-soluble matrix, containing defined numbers of MP particles or mass contents. These can be solved in water and handled as reference materials in drinking water without additional sample preparation. These samples are measured using mass-based methods as thermo-analytical methods like thermal desorption-gas chromatography-mass spectrometry (TED-GC/MS) or pyrolysis gas chromatography mass spectrometry (Py-GC/MS) and using number-based methods as vibrational spectroscopy like Infrared and Raman after filtration. Our aim was to develop a standard operating procedure to measure MP in complex matrices and to harmonise and compare different detection and characterization methods. These matrices require treatments to show a reliable quantification and identification of MP. We selected baby milk powder, as food matrix, which has a high content of water soluble and organic compounds. For the identification and quantification of small microplastics (SMPs; 10-100 µm) with TED-GC/MS and Py-GC/MS, a simple and fast pre-treatment with citric acid was applied. For the assessment of the number of SMPs, with vibrational spectroscopy (µ-Raman, µ-Infrared) and with electron microscopy (SEM), a multi-enzymatic digestion and a microwave alkaline hydrolysis was tested. As environmental sample, we selected surface water. The suspended particulate matter contained in the water has a high content of inorganic compounds. In this case, no sample preparation was used for the thermoanalytical methods and a sample preparation including density separation and oxidative digestion was tested to identify and quantify SMPs with vibrational spectroscopy. Depending on matrix and on the detection procedure, sample preparations influence significantly recovery rates. This work shows various analytical results and discusses technical aspects of the different procedures.

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3.22.T-04 A Paint Library of Plastic Particles (PLoPP): Fourier Transform Infrared Spectral Analysis of Paint Microplastics

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The degradation of paint is thought to be one of, if not the largest, source of microplastics in the

environment. However, researchers lack paint-specific spectral libraries that represent a wide variety of paint sources (e.g., roads, boats, buildings) to characterize paint microplastics in the environment and differentiate paint microplastics from non-paint microplastics. Here, we fill this gap by creating a Fourier transform infrared spectroscopy (FTIR) library of 263 spectra from 90 paints using attenuated total reflection (μ ATR-FTIR) called here a Paint Library of Plastic Products (PLoPP). The paints in the library are primarily used in seven sectors (architectural, automotive, consumer, general industrial, marine, road markings, and wood) and are in 15 colors and five appearances (glitter, gloss, matte, pearl, and semi-gloss). With the library, we also created a spectral analysis pipeline using machine learning to aid in the identification of paint vs. non-paint microplastics and to see if it is possible to trace back to paint sources. To assist in microplastic characterization as paints or non-paints, we also created a visual key for use with light microscopy. Using environmental microplastics, we verified the utility of these tools (visual key, ATR- μ FTIR spectral searches, and machine learning model classification) to characterize paint microplastics. This research creates tools to further our understanding of paint microplastics.

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3.22.T-05 Do Polymer Interactions Impact the Accuracy of Microplastic Quantification in Mass Spectrometry-Based Analysis?

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Microplastic (MP) pollution is an increasing global concern, with MPs detected in air, water, soil, and biota. Accurately quantifying these microplastics is challenging due to sample variability and the absence of standardized methods for analyzing polymer mixtures. A critical challenge is selecting reference materials that reliably replicate the decomposition behaviour of environmental polymer mixtures. Most studies focus on individual polymers to identify decomposition markers and develop calibration curves; however, this approach does not consider the complex interactions found in environmental samples where multiple polymers are co-pyrolyzed. These interactions may lead to secondary reactions, potentially introducing systematic errors in quantitative analyses. This challenge is particularly evident in thermoanalytical techniques such as TED-GC/MS and PY-GC/MS, where the co-pyrolysis of polymers can interfere with accurate quantification. This study investigates the impact of polymer interactions on thermal degradation fingerprints using thermogravimetric analysis coupled with gas chromatography and mass spectrometry (TGA-GC/MS). Various polymer combinations, including polystyrene (PS), polyethylene (PE), polyethylene terephthalate (PET), polyamide-6 (PA6), styrene-butadiene rubber (SBR), polyvinyl chloride (PVC), and polypropylene (PP), are analyzed with two different internal standards to evaluate their co-pyrolysis behaviour. Preliminary results indicated that increasing PS concentrations reduce the abundance of internal standard (poly(4-fluorostyrene)) monomer while promoting trimer formation, highlighting significant polymer interactions. Final results, expected by March 2025, aim to investigate specific fingerprints to prevent analytical errors caused by polymer interactions in MP quantification. Identifying polymer interactions and understanding their chemistry is crucial to avoid interferences and improve the accuracy of microplastic analysis.

3.22.P Matching Microplastic and Nanoplastic Reference Materials to Exposure Scenarios

3.22.P-Tu252 Polystyrene Nano- and Microplastics: Are We Using Relevant Particles for Exposure, Hazard, and Risk Assessment?

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During the past two decades there has been an exponential increase in the number of studies reporting on the toxicological effects associated with exposure to nano and microplastic particles (NMPs). Nevertheless, there is currently no commonly agreed mode of action (MOA) of these particles on human health and the environment. Neither has it been established whether all NMPs share the same MOA, or whether different NMPs of different size, shape, morphology, polymer chemistry, and surface reactivity act via different mechanisms. Collecting and characterising naturally occurring NMPs in sufficient quantities for research studies has so far proven to be impractical. Consequently, most studies have used monodispersed polystyrene microspheres (PSMs) as model particles. These PSMs differ considerably from NMPs that can be generated from commercial plastics, and from those that occur naturally in the environment, raising uncertainty as to the relevance of this research to human health and the environmental hazard and risk assessment. Our study of PSMs and generated NMPs has identified

properties of these particles that, if investigated in targeted research programs, may elucidate likely MOAs of both particle types and provide greater insight into the hazard and risk of naturally occurring NMPs.

3.22.P-Tu253 Optimized Reprecipitation Method for Nanoplastics and Small Microplastics as Test Materials

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Microplastics (generally defined as plastic fragments under 5mm) can be categorized by size, shape and polymer type. In particular, small micoplastics (sMP, <100 μ m) and nanoplastics (NPs, <1 μ m) are considered to pose high risk on organisms due to their bioaccumulation and capability to deliver adsorbing contaminants on NMPs which can cause combined toxicity. Although it is important to use well-characterized NMPs as test materials for investigating their effects, obtaining sufficient NMPs is challenging. In this work, the optimized reprecipitation method for synthesis of NPs and sMP was described. Reprecipitation method, in other words, non-solvent induced phase separation method, enables to prepare NPs and sMP with high yield and scale-up. For example, polyethylene (PE) and polypropylene (PP) are the most abundant polymer type in environment; however, the studies with PE and PP NPs and sMPs were very limited because of their lack of test materials, compared to PS NMPs which were commercially available with various sizes and homogeneity. Based on the optimized reprecipitation method, this work described three different sized (300~500 nm, 2~5 μ m, 20~50 μ m) and two different shaped (sphere and fragment) PP NMPs at a large scale (in grams) with high yield (90%). Moreover, homogeneously spherical PE NPs with 100~300 nm was also obtained the modified reprecipitation method. These NPs and sMPs have been used as test materials for their biological effects on various organisms such as zebrafish larvae, plant, and mouse. It is expected to be expanded to include additive chemicals on NPs and sMP in future works.

3.22.P-Tu254 Additive Manufacturing of Monodisperse Microplastic Reference Particles Through Micro Extrusion

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The environmental risks of microplastics are emphasized by their inclusion in the REACH Regulation of 2023 and further EU restrictions. While analytical methods exist for detecting microplastics, there is a critical lack of suitable reference materials for standardizing and validating these methods.

Producing microplastic references primarily relies on cryogenic grinding and screen fractionation, which comes with several challenges. For example, the use of liquid nitrogen and the fact that this method results only in dispersed fractions with limited size control. Additionally, a major requirement is to know the particle number, especially as conventional techniques only determine fraction weight. The distribution over multiple particle sizes prevent direct correlation between mass and particle count, necessitating time-consuming manual counting for precise numbers. Synthetic production of microplastic references faces similar problems with size distribution and unknown particle counts while being limited to few polymer types. All these limitations highlight the need for improved microplastic reference production methods. Thus, we present a cost-efficient and routinely capable method for the production of microplastic references using an additive manufacturing process by micro extrusion. The microplastic particles can be produced in exact numbers as well as in homogeneous size and shape. The process is suitable for all thermoplastics. Therefore, our method makes it possible to compare mass-based and number-based analysis methods for the first time. 10,000 particles can be easily produced within a few hours. We plan to fully automate the process and implement inline process control. The low error and reject rate mean that manufacturing time can be reduced and resources can be saved. So far, we have produced particles reproducibly in a size range of 300 to 1000 μ m from PLA, PCL, PMMA, PE and PA. In some cases, a minimum particle size of around 150 μ m has already been achieved. Another goal is to reduce the minimum particle size to below 100 μ m and to expand the range of polymers.

Our particles were already used in different environmental compartments for several months, for example, experiments in the Elbe River and vegetation plots, as well as in experiments on modeling microplastic transportation behavior within soil columns.

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3.22.P-Tu255 Influence of Fragmentation Techniques on the Biotic Aging of Polystyrene and Polypropylene Microplastics

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Microplastics are increasingly studied for their impact on the environment and preparation of tests materials are essential for laboratory experiments. However, laboratories often produce their own test materials by different methods, which can lead to significant differences in the physico-chemical properties of the produced microplastics. Recent research has revealed that the milling method used to produce microplastics can significantly influence their properties. In this study, we investigated how two commonly used milling methods by using ultracentrifugal and mixer mill affect the biofouling of microplastics. Polystyrene (PS) and polypropylene (PP) were milled under comparable conditions using both methods and then aged in natural stream water under laboratory conditions. The results showed that microplastics produced using different milling techniques age differently. In particular, PP microplastics produced with the mixer mill developed significantly more biofilm than those produced with the ultracentrifugal mill. But both PS and PP microplastics produced with the mixer mill exhibited significantly lower chlorophyll-a content and extracellular polymeric substances (EPS) compared to those produced with the ultracentrifugal mill. Furthermore, these differences in biofouling were reflected in the density changes after aging, with both PP and PS produced with the mixer mill having a lower density than those produced with the ultracentrifugal mill. These results highlight the important role of milling methods in determining the subsequent biofouling and physical properties of microplastics and emphasize the need for standardized preparation techniques to improve the comparability of different studies.

3.22.P-Tu256 Developing Reference Materials for Thermal and Vibrational Analysis of Microplastics

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Microplastics, ubiquitous pollutants in the environment, pose significant ecological and health risks. Accurate identification and quantification of microplastics are crucial for effective monitoring and mitigation strategies. Thermal analysis such as Pyrolysis-GCMS and vibrational analysis techniques such as μ FT-IR and μ Raman are powerful tools for microplastic characterization. However, these techniques require reliable reference materials to ensure accurate and reproducible results.

This presentation will discuss the development of a suite of reference materials specifically designed for microplastics, highlighting the neat microplastic reference materials (RMs) and certified reference materials (CRMs) for Py-GCMS for chemical identification of polymers and mass based quantification of microplastics, as well as soda tablets for μ FT-IR and μ Raman, highlighting tablet format for both chemical and particle morphological information, and number of particle based quantification. These reference materials will encompass a range of polymer types, sizes, and shapes, representative of common microplastic pollutants. The RMs and CRMs are rigorously characterized using a combination of techniques, including microscopy, spectroscopy, and chromatography.

The development of these reference materials will significantly enhance the accuracy and precision of microplastic analysis, enabling more reliable monitoring and assessment of environmental contamination.

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3.22.P-Tu257 Production of a 1-10 μ m Polypropylene Reference Material for Development of Analytical Methods

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Reference materials (RMs) are needed for harmonisation and validation of microplastic (MP) and nanoplastic extraction and analysis approaches, for the development standardised methods and for (eco)toxicity assessment. From an analytical chemical perspective, plastic particles in the size range 1-10

μm represent a specific challenge; most commonly used MP analysis techniques struggle with particles $<10\ \mu\text{m}$, whilst common NP analysis techniques struggle with particles $>1\ \mu\text{m}$. Plastic particle RMs in the 1-10 μm size range are important for the development and optimization of analysis techniques. Here, we present a method for the production of polypropylene (PP) MP in the target size 1-10 μm with an irregular morphology. PP pellets (100 g) were placed in 100 mL of ethanol and subjected to milling by UltraTurrax in an ice bath. The resulting mixture was filtered on 500 μm , 90 μm and 45 μm filters. The material $>500\ \mu\text{m}$ was milled again with the UltraTurrax and re-filtered. The $<45\ \mu\text{m}$ fractions were combined and further filtered using a 10 μm macroporous silicon filter. The filtrate was then re-filtered over a 1 μm macroporous silicon filter. The particles were re-suspended in ethanol and transferred to a preweighed vial, where the ethanol evaporated off. The final yield of 1-10 μm PP particles was 18.29 mg. The stock 1-10 μm PP material was re-suspended in MilliQ water and maintained in aqueous dispersion by addition of 0.025 wt% of BSA. Sub-samples of the PP dispersion were subject to imaging scanning electron microscopy (SEM), laser diffraction (LD), μRaman spectroscopy, MADLS, particle tracking analysis, TED-GC/MS and pyrolysis GC-MS analysis for physicochemical characterisation. SEM imaging confirmed the irregular morphology of the PP particles. The RM was tested according to ISO Guide 35:2017 with $n = 10$ on one property of interest, here particle size distribution. The PP particle suspension passed the homogeneity control with a particle size range between 300 nm to 7 μm , and a mean particle size of approximately 800 nm. Stability control will be performed again after 3 months. The results demonstrate that the top down production method is capable of producing irregular shape plastic particles that could be used for the development of analytical techniques targeted at MP $<10\ \mu\text{m}$. Furthermore, an irregular-shaped PP RM $<10\ \mu\text{m}$ offers the potential to close a current gap in MP (eco)toxicological assessment.

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3.22.P-Tu258 Impact of UV-Induced Aging on Microplastic Composition: Identification Challenges, and Implications for Environmental Monitoring

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Natural weathering processes of microplastics (MPs) not only lead to the production of secondary smaller size particles but may also alter the structure of polymer chains. These changes often consist in the increase of the oxygen contained functional groups such as carbonyl and carboxyl groups. Moreover, the aging process alters the physico-chemical properties of MPs, facilitating the leaching of additives and degradation products, potentially posing ecotoxicological risks. Furthermore, degraded polymers can present difficulties in their identification due to a poor match with the spectra of the pristine polymers. This could therefore complicate monitoring plans for MPs due to their misidentification. The aim of this research was to evaluate the differences in aged versus unaged MPs produced by mechanical fragmentation techniques starting from daily-used plastic products by using different techniques. A total of 5 true-to-life MPs (white and blue PS, PET, PVC, PA66) were photo-oxidized for 8 weeks in a UV-equipped furnace box system. Aged MPs were studied and compared with the unaged ones. Samples were analyzed by Py-GC-MS in double shot mode (thermal desorption followed by pyrolysis) to monitor whether the aging processes altered the chromatographic fingerprints. Moreover, untargeted analysis was performed using LC-HRMS (Orbitrap) on sample extracts, using Methanol as extraction solvent. Acquisition was performed both in positive and negative ionization. Chromatograms were then screened by Compound Discoverer 3.3 SP. The first differences observed were a change in color and a reduction in electrostatic behaviour between unaged and aged samples, particularly for PET and PS. The analysis of pyrograms revealed the presence of changes in some of the analyzed samples. For example, PVC microparticles were affected by the photodegradation in terms of number of pyrolyzates in aged samples in respect to unaged ones and in terms of different substances found. Indeed, some chemicals were only present in aged fragments rather than in unaged. This was also confirmed by untarget analysis, where 2-Ethylhexanoic acid and Dodecanedioic acid, used as plasticizers, were only detectable in aged samples. Since the existence of degradation products could be considered an indicator of the state of deterioration,

additional Py-GC-MS databases are required for identifying polymer-related pyrolytic products of environmental alteration processes.

3.22.P-Tu259 Experiments on Fragmentation and Abrasion of Plastics by Sediments and Impacts on Accelerated Chemical Leaching

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Plastic waste, ubiquitous in natural environments worldwide, accumulates over time and becomes an integral component of sedimentary systems such as rivers, beaches, and the deep seafloor. Within these systems, plastic items are transported alongside natural sediments, undergoing abrasion and fragmentation due to mechanical forces from their interactions with the sediments. These processes not only generate micro- and nanoplastics but also enhance the release of chemicals by increasing the surface area available for leaching. The accumulation and progressive fragmentation of plastics, combined with the release of hazardous chemicals, contributes to the "plastic toxicity debt," posing significant risks to future generations. However, the rate and extent of plastic fragmentation in sedimentary systems, as well as its interaction with chemical leaching, remain largely unknown, making it challenging to assess the ecological risk associated with this toxicity debt. To address these gaps, this study investigates the fragmentation of plastics during sediment transport and explores the correlation between the degree of fragmentation and chemicals leaching. Laboratory experiments were conducted to simulate plastic fragmentation mechanisms by abrading polystyrene cubes through mechanical interaction with sediments in freshwater. The experiments varied sediment parameters, such as grain-size and grain angularity, as well as the duration of abrasion. During the experiments, the samples underwent abrasion, shedding microplastics. Optical and electron microscopy analyses revealed a correlation between sediment parameters and the degree and type of abrasion over time. The generated microplastics were isolated and analysed for size distribution, with coarse sediment producing microplastics with a coarser size distribution compared to medium sediment, likely due to a flakier abrasion mechanism resulting in larger plastic fragments. In addition, preliminary chemical analyses of the fluid phase suggested that the concentrations of organic and inorganic components increased with increasing plastic abrasion. This study highlights the feasibility and potential of combining abrasion experiments with leachate analysis to investigate the relationship between plastic fragmentation and chemical leaching. The findings provide valuable insights for a comprehensive hazard assessment of plastic pollution, essential for future environmental planning and mitigation strategies.

3.22.P-Tu260 Preparation and Characterisation of Representative Micro and Nanoplastic Test Materials

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Micro- and nanoplastics (MNPs) are ubiquitous, however suitable risk assessments are still lacking due to poor quality control, the lack of standardised approaches to particle characterisation and environmental analysis, and the use of test materials that do not reflect those found in the environment for toxicity testing. Currently used test materials are often monodisperse polystyrene microbeads which are unrepresentative of environmental MNPs due to the perfectly round morphology, lack of diversity in plastic type and use of surfactants. A set of well-characterised test materials that are more representative of environmental MNPs would greatly help with the development of relevant risk assessments.

In this work, we aimed to develop a method for preparing test materials that are more environmentally representative. Such materials should: a) represent different, widely used plastics; b) possess a fragment morphology; and c) have representative bulk and surface chemistry. To achieve this we developed a four step protocol that involved coarse milling, (cryogenic) ball milling, sedimentation and sieving. The plastics used were commercial grades and particles were suspended without surfactants to ensure relevant chemistry. We successfully demonstrated the suitability of this protocol by preparing multiple different size fractions of both PP and PVC. These particles were also comprehensively characterised to provide information on contamination, chemical bonding, size and morphology.

Suspension of MNPs without surfactants is not without challenges as in water the particles have limited physical stability and will agglomerate whereas in organic solvents certain plastics may have limited

chemical stability, exhibiting either dissolution or swelling. We demonstrated that by combining descriptors for physical and chemical stability (zeta potential and Hansen Solubility Parameters, respectively) it is possible to predict suitable particle suspension media. Current work is focused on increasing the environmental relevance of particles by including weathering processes such as UV-ageing that may play an important role in the behaviour, uptake and (eco)toxicity of MNPs.

We believe that the particle preparation protocol developed, along with the insights into surfactant-free suspension of particles, can advance the field by enabling research groups to study the effects of MNPs using more environmentally relevant materials thus leading to improved risk assessments.

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3.22.P-Tu261 Processing Tablets as Reference Material for Microplastics Quantification in Environmental and Food Matrices

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Analytical detection methods and techniques to identify and quantify microplastics in environmental and food matrices are numerous but validated and standardized methods are currently lacking. To close this research gap, representative reference materials (RMs) and sample preparation approaches are urgently needed. Homogeneity and stability are two crucial characteristics for the certification process of the reference materials. For this purpose, we aim to present ways to prepare reference materials containing very low masses of close-to-reality plastic particles with broad size distribution (10-100 µm) that are homogeneously distributed in tablet-to-tablet variation and stable over time. RMs were prepared by choosing polypropylene (PP) as polymer due to food and environmental relevance and a water-soluble matrix composed by lactose and polyethylene glycol (PEG). The PP powder was obtained by cryogenic milling from pellets and sieved afterwards to isolate size fraction within a certain range focusing our analysis on the size fraction < 50 µm. For the production of homogeneously distributed PP particles in the individual tablets, polypropylene (< 50 µm), lactose and PEG were added step by step and mixed in between before tableting. In order to develop a validated method to produce RMs, four different approaches will be carried out by changing two variables: (i) matrix size distribution (sieved or unsieved to the same sizes as PP) and (ii) homogenization mixer instrument (Thumble mixer or Y mixer). The results of polypropylene particle size distribution measurements by laser diffraction show that most particles are in the wished size range between 10 and 100 µm with a D50 of 48.382 ± 0.045 µm, therefore the powder is homogeneous. Thermogravimetric analysis measures the mass loss of PP and its decomposition temperature; the TGA curves obtained from the first tests confirm that the lost mass between 350 and 490 °C corresponds to the concentration of PP present for each tablet.

This work is based on the ISO Guide 30:2015 and ISO Guide 35:2017 to fulfil the criteria set in future standards ISO/DIS 16094-2 and ISO/DIS 16094-3. The property of interest is the mass detected by thermogravimetric analysis (TGA) but the future perspective of this project also aims at integrating spectroscopic analyses (Raman microscopy or µFTIR) to obtain particle numbers by size class and chemical identification as informative values.

3.22.P-Tu262 Advanced Approach to Collect Small Microplastics in Environmental Water Samples Using Automatic Sample Preparation Technique

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The occurrence of microplastics (MPs) and nanoplastics (NPs) in the environment is a global issue that requires the collaboration of multiple stakeholders to ensure the life cycle of plastics is properly monitored and managed. Most international efforts have focused so far on the standardization and validation of detection techniques, including Raman and FTIR spectroscopy and Py-GCMS. However, now that regulations for MPs are being developed and more environmental laboratories are starting their routine analysis, effective sample preparation is crucial for their success. Current approaches for eliminating organic content from samples or for separating inorganic materials rely on manual digestion and density separation, and the performance of these steps depends on the operator's proficiency. To obtain more accurate data between laboratories, the homogenized method must be required. In addition, converting from manual to automatic operation would be a solution to improve the accuracy in the data. The

automatic preparation device, providing a universal sample preparation process suitable for the follow-up analysis by Raman, FTIR or Py-GCMS, has been available. But it still has some challenges in the treatment of smaller microplastics. This presentation will provide some experiments data using automatic preparation device for isolating smaller microplastics in environmental water samples.

3.22.P-Tu263 Extracting and Quantifying Nanoplastics in Food Matrices

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Due to their smaller dimensions and specific colloidal properties, small microplastic (sMPs; 1-10 µm size range in this work) and nanoplastics (NPs; < 1µm in this work) are likely to cross biological barriers, increasing the potential for accumulation and the possibility of negative effects on living organisms. Recent studies have highlighted the potential presence of sMPs and NPs in bottled drinking water and in milk powdered infant formulas, raising concerns about exposure during early development.

Here, we address the quantification of NPs in food-related samples such as drinking water and milk, establishing the lower detection limit as a function of particle size and aiming to achieve precise and reliable characterization.

In the framework of the Plastic Trace Project (plastictrace.eu), sub-micron and nano-sized plastic particles, representative of common industrial polymers like polyethylene (PE) and polypropylene (PP), were produced and characterised. These materials, designed to mimic real-life particles in terms of size, shape, and aging, were then spiked into bottled drinking water and infant formula. This work focuses on developing optimized sample preparation strategies for the extraction of NPs from complex food matrices, with an emphasis on maximizing particle recovery rates while preserving the original characteristics of the particles. The stability of nanomaterials spiked in these complex matrices is also addressed. Different strategies for the enrichment of nano-polypropylene (nanoPP) dispersed in bottled drinking water are currently being tested. This includes (i) nanoscale filtration of nanoPP and recovery in surfactants assisted by probe ultrasonication; (ii) Cloud Point Extraction (CPE) methods based on Triton X-45, and (iii) freeze-drying. Multi-detector Asymmetrical Flow-Field Flow Fractionation (MD-AF4) is used for the separation and characterization of nanoPP before spiking and after extraction from the matrices. Fit-for-purpose fractionation methods for separation of NPs dispersed in milk matrices are also currently studied to provide an alternative to more invasive extraction methods.

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3.22.P-Tu264 A Roadmap for Harmonized Microplastic Analysis in Textile Wastewater Treatment Plants

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The growing concern over microplastic pollution in aquatic environments highlights the need for harmonized methodologies, particularly for complex matrices such as textile wastewater. Current approaches lack harmonization, leading to inconsistent results that hinder reliable assessments. This study proposes a robust roadmap for the extraction and analysis of microplastics from textile wastewater. The framework begins with optimized extraction methods tailored to complex wastewater matrices, ensuring minimal interference from co-contaminants. Analytical pathways include optical microscopy visual inspection, Fourier-transform Infrared spectroscopy, and Pyrolysis GC-MS for identification and quantification, supported by comprehensive uncertainty budget estimation. Key elements of the protocol involve the systematic analysis of blanks, evaluation of subsampling techniques, and the use of replicates to ensure data reliability. Furthermore, an interlaboratory comparison among multiple facilities validates

the developed protocols.

The focus on harmonization across these steps aims to establish reproducible and comparable results, thereby advancing the field of microplastic research. This study's roadmap provides critical insights into improving protocol harmonization and ensuring robust data generation. These findings are not only essential for understanding microplastic pollution from textile wastewater but also provide a foundation for policy-making and mitigation strategies. The methodology's adaptability further enhances its relevance for broader environmental applications.

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3.22.P-Tu265 Developing a Protocol for Accurate Qualitative Assessment of Paint-Derived Microplastics in the Environment: Density and FTIR Analysis

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Paint particles, particularly those derived from antifouling and other marine coatings, are a significant but under-recognized source of microplastic pollution in the environment. Due to their complex composition of polymers (binders), pigments, and additives, paint particles exhibit diverse physical and chemical properties, such as higher densities compared to pure polymers, which complicates their identification and environmental analysis. This research aims to address these challenges by improving the qualitative assessment of microplastics derived from paint through two critical approaches: density analysis and FTIR spectrum interpretation.

This study investigates the environmental fate of paint particles by measuring their densities and predicting sedimentation behavior using Stokes' Law. Comparisons were made between pure polymers, commercially available paint particles, and those sampled from environmental matrices, revealing density variations due to pigments and additives. Additionally, FTIR spectra of paint particles were analyzed to identify functional groups and molecular motions, focusing on fingerprint regions that distinguish paint particles from other microplastics.

Results indicate that paint particles have significantly higher densities than standard polymers, often leading to their omission in density-based separation processes (e.g., 1.6 g/cm³ cutoff) commonly used in microplastic research. Furthermore, FTIR analysis identified unique peaks corresponding to polymers such as acrylate, rosin, PDMS, epoxy, urethane, and alkyd, facilitating the development of a protocol for the accurate identification of paint-derived microplastics.

This study emphasizes the importance of integrating density-specific methods and FTIR fingerprinting to enhance the detection and characterization of paint particles in environmental samples. By addressing critical gaps in current methodologies, these findings provide a foundation for more accurate monitoring and risk assessment of paint-derived microplastic pollution.

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3.22.P-Tu266 Development of Analytical Methods for Polymers (Unique Plastics) That Cannot be Analyzed by Hydrogen Peroxide Oxidation Treatment

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Microplastics (MPs) are a global environmental concern, with ongoing research improving our understanding of their distribution and characteristics. However, existing methods for accurately detecting all types of plastics remain insufficient, particularly for biodegradable plastics and those with unique properties, such as Polyvinyl Alcohol (PVAI) and Super Absorbent Polymers (SAP). PVAI, widely used in products like detergent pods, has seen its global production grow from 3.7 million tons in 2018 to 4 million tons in 2022. While considered biodegradable, its high water solubility raises questions about persistence in aquatic environments. Studies like Rolsky et al. (2021) indicate PVAI degradation requires specific conditions, such as specialized microorganisms, optimal temperature, pH, and oxygen levels. In their absence, PVAI may persist longer than anticipated.

SAP, with an annual production of 3 million tons, is non-biodegradable but exhibits high water absorption

capacity, which decreases in saline conditions. SAP distribution in aquatic environments remains poorly documented. Existing methods face challenges in detecting PVAI and SAP. For example, at the standard 55°C used in hydrogen peroxide oxidation, over 99% of PVAI becomes undetectable by micro-FTIR analysis. SAP particles often fragment during analysis, leading to underestimation of particles smaller than several hundred micrometers. Thus, traditional methods may underestimate these plastics in environmental waters.

In river surveys conducted in Japan (2022-2023), a 300/350 µm plankton net collected 2,897 MPs, of which 22 were identified as PVAI via FTIR analysis. Follow-up studies using a 10 µm net detected no PVAI and few SAP particles. These results contrast with reports of PVAI and SAP as macroplastic debris on beaches, raising concerns about their degradation into smaller particles and behavior in aquatic environments. Like other plastics, PVAI can adsorb pollutants, posing ecological risks. This highlights the need for improved monitoring of PVAI and SAP to assess their environmental impact.

This study introduces a novel method for quantifying PVAI and SAP in environmental waters, addressing limitations of traditional approaches. By providing a more accurate assessment of their concentrations, this method enhances understanding of their distribution and impact.

3.22.P-Tu267 Innovations in Microplastic Measurement: A Pyrolysis-Gas Chromatography/Mass Spectrometry for a Polyethylene Terephthalate Reference Material

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In the framework of the PlasticTrace project, in which a reference material for PET microplastics will be proposed, a method using pyrolysis and gas chromatography coupled with mass spectrometry detection (Py-GC-MS) is developed to quantify the mass of PET microplastics (10-100 µm) in tablets at two different concentration levels.

The overall aim of this project is to develop international metrological capacity that enables the traceable measurement and characterization of SMPs and NPs in environmental and food samples and the production of suitable reference materials, according to the metrological requirements (<https://plastictrace.eu/>).

The proposed method consists of dissolving the tablet containing the PET microplastics in milli-Q water and filtering the solution using a glass microfiber membrane 13 mm filter size, 1.6 µm pore size (Whatman). After filtering and several washes to ensure complete dissolution of the tablet and recovery of all PET microplastics, the filter is removed, folded, and placed in a capsule of the pyrolyzer for direct Py-GC-MS analysis.

Two main markers were selected for PET quantification: vinyl benzoate and benzoic acid, with consistent concentrations found for both at high PET concentrations (400 µg) (24% RSD, n = 17), but interferences with the matrix and vinyl benzoate were found at low concentrations (17 µg PET), with only benzoic acid being valid as a marker in this case (22% RSD, n=10).

As an alternative to the quantification of PET microplastics in tablets at low concentrations, derivatization is proposed, for which tetramethylammonium hydroxide solution (TMAH) is added directly into the pyrolysis cap. The PET marker in this case is dimethyl terephthalate, with which good results have also been found (19.6% RSD, n = 10).

The proposed method is robust, simple, with high automation and very little sample handling and treatment, and allows the analysis of PET microplastics (10-100 µm) in tablets with excellent accuracy and precision even at very low concentrations.

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3.22.P-Tu268 Identification of Microfibers Released from Textiles in Simulated Accelerated Washing Using International Standards: Implications for Comparative Analyses

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Microplastics, including plastic microfibers, are increasingly recognized as a significant environmental issue. Their ubiquitous presence in various environmental matrices, coupled with their complex composition and morphology, implies the need for standardized methodologies for their accurate detection and quantification. This study aimed to contribute to the development of such standardized approaches by investigating the release of microfibers from different fabrics during simulated washing processes, adhering to established international standards.

By adopting these standardized protocols, researchers can generate reliable and comparable data on microfiber release, enabling a more comprehensive understanding of the factors influencing their prevalence and environmental fate. This knowledge is crucial for developing effective strategies to mitigate microplastic pollution and protect ecosystems.

Our findings emphasize the importance and requirement of careful data analysis, particularly when relying on automated software-based identification techniques. While these techniques offer potential advantages in terms of efficiency and high-throughput, they may introduce biases and inaccuracies, especially for complex samples. Therefore, a combination of analytical techniques, including manual verification and complementary methods, is recommended to ensure accurate and reliable results.

By promoting the use of standardized methodologies and critical data analysis, this study contributes to the advancement of microplastic research and the development of evidence-based solutions to address this emerging environmental challenge.

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3.22.P-Tu269 Approaches and Strategies for the Detection and Quantification of Nano and Microplastics by Single Particle Inductively Coupled Plasma Mass Spectrometry

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Plastic is the most prevalent type of marine debris found in our ocean and lakes. Plastic debris can come in all shapes and sizes, but those that are less than five millimeters in length are called microplastics.

Microplastics enter the environment from a variety of sources, environment weathering of large plastic items, additive manufacturing used in cosmetics and release from consumer products.

Currently FT-IR microscopy is the most common approach used for microplastic research. Here we show that single particle inductively coupled plasma mass spectrometry (SP-ICP-MS) can be utilized for the analysis of micro-sized plastics by monitoring carbon isotopes. We discuss the use of specific introduction parts, to ensure an even transport efficiency of both dissolved and micron-sized particles into the plasma, as well as strategies using specific gases and cell conditions to obtain the best detection limits. This approach can be used for fast scanning of microplastic particles in environmental matrices or consumer products without needing to concentrate or alter the samples matrices.

3.22.P-Tu270 Detection of Nanoplastics in Liquid Using Surface-Enhanced-Raman-Spectroscopy-tags

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Nanoplastic detection (defined here as plastic particles smaller than 100 nm) has become critically important due to its widespread presence, persistence in various ecosystems, and potential toxicity to wildlife and humans. Despite this concern, the analytical techniques available for detecting and quantifying nanoplastics, especially at environmentally relevant concentrations ($\mu\text{g/L}$ ng/L), remain significantly limited.

Surface-enhanced Raman spectroscopy (SERS) has shown promising potential for achieving the necessary sensitivity to detect nanoplastics below the conventional Raman resolution limit (~ 500 nm), even at concentrations as low as ng/L. However, many existing SERS approaches, which rely on aggregated plasmonic particles, suffer from weak reproducibility and the inability of quantification in environmental contexts.

To address these limitations, we adopted a strategy that employs SERS tags to indirectly detect nanoplastics. By conjugating gold nanostars with a reporter molecule with an inherently strong Raman signal, we are able to measure signal changes of the reporter molecule in the presence of plastic particles. This signal change depends on the plastic concentration and, therefore, allows us to quantify the nanoplastics in an artificial aqueous sample at low concentrations. This method could enhance our ability to detect nanoplastics at realistic concentrations in natural freshwater and marine environments and more complex aqueous environmental matrices.

3.22.P-Tu271 Advancing Microplastic Research through True-to-Life Micro- and Nanoplastics for Environmental and Biological Studies

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Micro- and nanoplastics have become significant environmental pollutants, posing potential threats to aquatic ecosystems and human health. Despite growing concerns, there is a lack of harmonized methods for accurately identifying and assessing the impact of these particles. A major challenge in developing more relevant test microplastics is finding processes that closely replicate their environmental counterparts. To address this, we have developed "true-to-life" micro- and nanoplastics, along with comprehensive analytical protocols for their identification, characterization, and impact assessment. This approach aims to enhance our understanding of the sources, fate, and effects of these particles. In particular, we explored and compared three mechanical fragmentation techniques for preparing true-to-life materials, examining how each method affects the morphology and structural properties of the resulting microplastics. Through centrifugation protocols, we successfully isolated nanosized fractions from micrometric plastic powders. Nanoplastics were then characterized using both chemical and imaging techniques, revealing unique morphological features for each nanoplastic type. This diversity in particle characteristics led to distinct bio-interactions with proteins and environmental compounds. Lastly, we investigated the dynamics of microplastic uptake, accumulation, and egestion in biota by exposing mussels to true-to-life microplastics. Our findings underscore the importance of employing materials with greater environmental relevance, bridging the gap between laboratory experiments and real-world observations.

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3.22.P-Tu272 Investigation of Fluorescent Polystyrene Uptake in Caco-2 Cells Using Microplate Readers

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Microplastics (MPs) are a growing concern due to their potential impact on human health, including their uptake by cells and accumulation in tissues. Detecting MP uptake by human cell lines is critical for understanding exposure pathways, but current techniques such as transmission electron microscopy, flow

cytometry, and Raman microscopy are labor-intensive, time-consuming, and require specialized skills. This study aims to develop a fast and user-friendly detection method using a microplate reader, which could offer a high-throughput alternative for studying MPs-cell interactions. Fluorescent polystyrene (PS, 1 µm) was selected due to its prevalence in consumer products and the ease of size control during synthesis. Caco-2 cells were incubated with different PS concentrations (0, 10², 10³, 10⁴ particles/mL) for 24 hours, followed by washing, fixing, and measuring fluorescence using a microplate reader. Results showed a correlation between increasing PS concentrations and fluorescence intensity, but the microplate reader failed to detect uptake at the lowest concentration (10² particles/mL), as values were indistinguishable from the control group (0 particles/mL). These findings suggest that microplate readers can provide a fast and efficient means of detecting MP uptake by cells at higher concentrations (>10³ particles/mL), offering a practical tool for high-throughput studies. This method advances the field by simplifying detection protocols, making it more accessible for broader use, and contributing to a better understanding of MP exposure risks and mitigation strategies.

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3.22.P-Tu273 Leaching of Plastic Additives from Custom-Made Polymers into Artificial Gut Fluids

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Microplastics are pervasive in the environment, including their presence in the food web. Humans can ingest microplastics through beverages and food, and are exposed not only to the microplastic particles themselves, but also to the plastic additives. These additives can leach into human intestinal fluids during passage of the gastrointestinal tract, where they may be transformed and bioaccumulate, thus posing a risk to human health.

As the composition and concentration of additives in plastic products are generally not disclosed, this study used custom-made polymers with known and realistic additive mixtures. The additives were chosen to cover different categories such as UV stabilizers, plasticizers, and flame retardants. We used UV-weathered plastic as UV-light may cause surface alteration, degradation and structural modification of the plastic.

The study investigated material-specific leaching across different polymers and additives, comparing weathered and unweathered plastics and varying incubation times. By utilizing plastics with known additive concentrations, the extent of leaching was determined, providing critical insights into plastics with unknown compositions.

To simulate human digestion, microplastics were exposed to artificial gut fluids representing saliva, gastric juice, duodenal juice, and bile, with conditions reflecting physiological temperature, pH, and residence time in each digestive phase. To provide a more holistic understanding, a simplified human intestinal microbiota (SIHUMix) was applied followed by metabolome and proteome analysis. Finally, the additives were extracted using hexane, and their concentration in the solution was measured.

These findings aim to improve risk assessments and inform regulatory agencies regarding plastic additives, particularly with respect to human gastrointestinal exposure.

3.22.P-Tu274 Influence of Additives on the Abiotic Degradation of Polyethylene Plates

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Secondary microplastics in the environment are mainly caused by the ageing of macroplastics. However, the extent to which this ageing is influenced by the addition of additives still needs to be clarified.

Accelerated abiotic weathering was used to investigate the mechanisms that lead to the degradation of additivated, semi-crystalline low-density polyethylene (LDPE). For this purpose, LDPE plates were produced with different, realistic concentrations of additives and weathered in a test chamber. Oxidation products from photooxidation and fragmentation processes were detected on the surfaces of the LDPE plates. Analyses of the aged plates clearly showed the influence of the additives on the ageing of the macroplastics. Based on these results, it should be possible to model the degradation process of additivated plastics in the future.

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3.22.P-Tu275 Investigation of the Photo-Oxidation and Casein Interaction of Polystyrene Microplastics

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Ultraviolet (UV) radiation might cause photo-oxidation of plastic polymers driven by free radicals. Comprehending the effects of UV-induced photo-oxidation on microplastics (MPs) is essential for assessing their ongoing environmental impact and interactions with other molecules. In our study, 1 μ m polystyrene (PS) MPs were exposed to 36 W UV-C light for 10 days in a dry state and subsequently analysed for interactions with casein to explore the altered behaviour of photo-oxidized MPs. Chemical characterisation of samples (casein-interacted pristine MPs and casein-interacted UV-aged MPs) was conducted using optical photothermal infrared (O-PTIR), obtaining infrared (IR) spectra. This novel method enables the detection of submicron particles with 500 nm spatial resolution and addresses some challenges posed by Raman and Fourier Transform Infrared Spectroscopy (FTIR). Our preliminary result showed that characteristic peaks related to carbonyl groups (~ 1743 cm⁻¹) exhibited slight shifts after 10 days of UV exposure. Following casein interaction, protein-related peaks between 1600-1700 cm⁻¹ were also observed in the IR spectra. Scanning electron microscopy (SEM) images showed the altered surface after UV exposure and the formation of a protein corona following casein interaction. Dynamic light scattering (DLS) analysis revealed that UV exposure led to the formation of smaller PS MPs of approximately 150 nm in size compared to untreated samples. Furthermore, the particles exposed to UV had a diameter about 100 nm larger after interacting with casein compared to unexposed particles, although this difference was not statistically significant. This could be attributed to the formation of functional groups on the particle surface due to photo-oxidation. Zeta potential analysis indicated that the zeta potential of both untreated and UV-treated samples closed the zeta potential value of casein after the interaction. Moreover, atomic force microscopy (AFM) analysis showed that the stiffness of PS MPs decreased after casein interaction. In conclusion, our preliminary findings suggest that UV radiation changed both the physical and chemical properties of 1 μ m PS MPs, and this alteration might affect the protein interaction of the particles. These insights are critical for understanding the environmental fate and biological impact of aged microplastics, particularly in systems where protein interactions play a significant role, such as food systems and human health.

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3.22.P-Tu276 Bioprocessing of Printed Circuit Board Microplastics

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Electronic waste (e-waste) is the fastest-growing waste stream globally, comprising a complex mixture of hazardous materials, including metals, plastics, flame retardants, and plasticizers. Despite advances in recycling technologies, challenges persist in sorting and decontaminating the polymer fractions (over 27% of e-waste), leading to their frequent unregulated disposal. In this context, building up approaches with increased sustainability towards processing these plastic constituents becomes essential, ensuring both efficient resource recovery and mitigation of environmental impacts.

Microorganisms such as fungi have demonstrated a broad ability to degrade and metabolize a variety of plastics. Still, their use for the biodegradation of e-waste plastic fractions has yet to be attempted. In this context, this study explored the potential of the fungus *Penicillium brevicompactum*, a species previously found to have plastic biodegradation potential (including polymers used in electronics) for the biodegradation of the plastic fraction of printed circuit boards (microplastics 1-2 mm in size). Exposure to *P. brevicompactum* over 28 days in a liquid medium, under stirring, was associated with a concurrent increase in the number and decrease in the size of microplastic particles and mass reductions of up to 75% (specifically in the first 14 days). A further indication of this fungus capacity for PCB microplastic

biodegradation is the occurrence of chain scission and oxidation events, as assessed through attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy. On the other hand, regarding fungal biomass, absorption intensities were observed in regions attributable to functional groups associated with carbohydrates (assessed through ATR-FTIR), which signals these microplastics' potential use as a fungus carbon source.

These results highlight the promising role of fungi such as *Penicillium brevicompactum* in the biodegradation of e-waste-derived microplastics, which further represents a basis for research into broader and more sustainable e-waste bioprocessing strategies.

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3.22.P-Tu277 Rethinking Biofilms on Plastics as Environmental Defenders

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Plastic pollution introduces a range of harmful chemical additives into natural environments due to physicochemical aging and subsequent leaching processes. However, the role of biofilms microbial communities that form on plastic surfaces remains largely unexplored in this context. This study proposes that biofilms may act as a natural barrier, capturing and potentially metabolizing these additives before they disperse into the environment, while also protecting the plastic itself from environmental degradation. Such interactions could fundamentally change our understanding of plastic persistence and toxicity in ecosystems.

To test our hypotheses, we conduct controlled experiments exposing consumer plastics of various polymer types to natural water to induce biofilm formation. Plastic samples are maintained in both biofilm-supportive and biofilm-inhibited environments, enabling direct comparison. Plastics from both sets are then exposed to UV radiation, to evaluate the potential protective role of biofilms on plastics against photoaging.

The fingerprints of biochemical alterations from the uppermost plastic surface along particle's depth is characterized using Raman micro-spectroscopy, with a depth resolution down to 5 µm. Concurrently, alterations in surface chemistry, including wettability, zeta potential, and surface energy, are also analyzed. Changes in surface morphology are monitored by scanning electron microscopy and atomic force microscopy coupled with Raman micro-spectroscopy. To characterize and measure leachates and potential metabolites generated within the biofilm, we employ gas chromatography and liquid chromatography coupled with mass spectrometry on nominal and high resolution mode.

The findings from this study are expected to provide valuable new insights into plastic risk assessment, promoting a more realistic evaluation of plastic's environmental impact and fate. By reframing biofilms as potential defenders of both the environment and plastic materials, this research aims to enhance our understanding of plastic pollution and its mitigation within natural ecosystems.

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3.23.A Plastics in Terrestrial Ecosystems: Balancing Applications with Impacts, Fate and Hazards

3.23.A.T-01 (Micro)Plastics in Soils: From Sampling Strategy to Source Identification and Conclusions for Regulation

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(Micro)plastics are ubiquitous in our environment, including the soil. Reliable monitoring data help to

understand input pathways, to assess potential risks, and to derive recommendations for action. In the context of soil protection, robust cost-effective sampling and analytical methods are needed. Within the project PLASBo "harmonised methods for plastics and microplastics in soils," we developed a soil sampling strategy for subsequent (micro)plastic analysis and evaluated a visual method for routine analysis of plastics >1 mm in an inter-laboratory comparison. Moreover, we generated a first comprehensive database on micro(plastics) in soils and identified major input pathways. Samples from 113 sites of different land use types across Austria (non-representative selection) were collected and analysed by visual inspection (>1 mm) as well as by micro-FTIR (0.05-1 mm), detecting the 10 most common plastic types. For 25 sites, tire wear was additionally analysed by TED-GC-MS. A complementary site characterization allowed identification of factors influencing the concentrations, sizes, shapes, and types of plastics identified.

The sampling strategy proofed fit for various land use types and can be used in combination with visual analysis to reliably quantify concentrations of large (micro)plastics (>1 mm) >10 n/kg. Visual analysis was found to be robust, with a low error rate, despite some impact of analytical experience.

Small microplastics (0.05-1 mm) were virtually omnipresent, with concentrations about 3 orders of magnitude higher (mainly $\sim 10^2$ - 10^4 n/kg) than for large (micro)plastics (mainly 1-10 n/kg). The concentrations decreased with decreasing use intensity. Impacts of fertilizer applications (sewage sludge, compost, manure, none) and plasticulture (e.g., mulch foils in vegetable farming) were reflected in (micro)plastic concentrations and compositions. Some hotspot sites were related to closeby emitters, and for roadside/commercial and leisure areas impacts of littering were evident. Tire wear was detected at 16/25 sites at concentrations of <1.1-61 mg/kg and coincided with the proximity of roads, urban areas, and sewage sludge application. For forests, deposition and the impact of the humuslayer on the transfer of microplastics into underlying mineral soils was evident.

The results provide a valuable basis for method harmonisation and the prioritisation of recommendations for action in the regulatory context of soil protection.

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3.23.A.T-02 Assessment of Sampling Approaches for Representative Quantification of Microplastics in Soil

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Microplastics (MPs) concentrations and accumulation in soils are highly spatially variable and are dependent on proximity to sources, land-use and natural processes. The entire analytical chain for quantifying and characterizing MPs in soils is challenging, particularly in terms of accurately capturing the variations of a heterogeneously distributed particulate contaminant, which is the foundation for all future steps in assessing MPs exposure. Current approaches for MPs sampling rarely consider the sampling design, matrix characteristics and the spatial representativeness of each sample. Using metal-doped MPs to more quickly assess sampling strategies by circumventing the time needed for traditional MPs analysis, we produced a reproducible and representative hypothesis-driven sampling framework from which MPs can be quantified from a diverse range of soil environments. MPs of different morphologies (fragments, fibers) and concentrations (0.001-0.1 %) were mixed into different model systems (100 x 100 x 5 cm and 40 x 40 x 30) at distribution patterns mimicking clustered or homogeneously polluted systems. We created a multi-factorial experiment to determine the most effective combination of the individual components (sampling design, frequency, sample mass, homogenization and separation techniques, as well as composite aliquot factors) to accurately represent different distributions and concentrations of MPs in soils. Initial results highlight that techniques to reduce a sample mass by an order of magnitude must have >90% recovery for integration in the sampling framework. Similarly, a higher composite aliquot factor of 5:1 (aliquots:composite) returned higher recover rates than direct analysis of individual samples, for both fragments and fibers. From 0.02-0.09 wt.% (MPs/soil), recovery rates of direct digestion were $91.3\% \pm 10.1\%$, compared to density separation ($85.7\% \pm 4.5\%$). However, when two density separation cycles were performed, recovery rates were comparable to direct digestion ($94.6\% \pm 3.1\%$). The development of this sampling framework is critical for the accurate assessment of MPs in different soil environments, which ultimately facilitates comparability of data between studies. Using this approach can provide guidance for MPs monitoring in soil, which is urgently needed for the accurate assessment of MPs

exposure in the terrestrial environment and to better understand the mechanisms governing MPs fate, mobility, behavior and impacts.

3.23.A.T-03 Fate of Microplastics Derived from Agricultural Plastics in Soil Systems: Insights from Mesocosm and Field Experiments

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Soils have been identified as one of the main environmental recipients of microplastic pollution, where the fragmentation of mulching films during and after use has been highlighted as an important source. Yet, little is known about the environmental fate of mulching film fragments once they enter soil ecosystems. This is important as, if microplastics remain in the soil, then the successive use of mulching films may lead to increasingly high concentrations over time. If they are instead effectively mobilised, then soils may act as a release pathway for microplastics to connected environments. This study addresses this critical knowledge gap by combining multiple experiments across different scales.

First, a series of mesocosm experiments were conducted using the CLIMECS (CLImatic Manipulation of ECosystem Samples) facility at Vrije Universiteit Amsterdam, which comprises 40 soil columns that simulate soil ecosystems and are individually controlled for different environmental conditions. Different treatments investigated included a range of microplastic concentrations, high and low watering regimes, and the presence and absence of earthworms. Insights from this experiment were further investigated under field conditions, as part of multi-season field plot experiments conducted in Finland, Germany, and Spain. Experimental plots were established in fields with known histories of plastic exposure. The experiments were maintained from the onset of the growing season in each country in 2022 until the end of the growing season in 2023. Samples were taken at the end of both growing seasons. Two polymer types (linear low density polyethylene and polybutylene adipate terephthalate), produced from conventional and biodegradable mulching films, respectively, were tested across both experiments. Microplastics were analysed in soil and earthworm samples to track particle fate.

Vertical transport of microplastics was noted, where the critical role of earthworms was revealed. High soil water inputs exerted a lesser but observable influence. Different earthworm species take up particles to a different degree. However, despite vertical migration, the majority of particles remain in the upper layers where they are more available for lateral transport processes. Together, these experiments help to identify important mechanisms governing microplastic fate in soils systems. These insights are valuable in assessing the sustainable use of mulching films in soil-food systems.

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3.23.A.T-04 Incorporation of Microplastics into Water-stable Soil Aggregates: Dependencies on Particle and Soil Characteristics

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Agricultural practices like irrigation, mulching, and fertilization are closely associated with the pollution of soils by microplastics (MPs). In these environments, MPs can be transported both vertically and horizontally, with the potential to ultimately reach groundwater or surface water sources. While vertical MPs transport has gained considerable attention, horizontal transport remains underexplored. In soil systems, MPs exhibit interactions with other particles and form aggregates. The incorporation of MPs into these aggregates might strongly change their later transport behaviour, particularly when these aggregates show water resistance (water stable soil aggregates, WSAs). This study attempted to understand the incorporation of MPs into WSAs with regards to both MPs and soil properties. To do so, polyethylene terephthalate (PET) and two BASF seed coating fragments were spiked at 0.5% (wt/wt) into a soil that was sieved to <250 µm. All materials contained rare metals to facilitate tracing. MPs were milled to 150

250 µm while PET was additionally milled to <63 µm. For one variant, PET fragments 150-250 µm were mixed with the same soil that was re-combined with the sand fraction (250-2000 µm). All soils were incubated in triplicates at 60% of their maximum water-holding capacity for 2 weeks. To provoke faster aggregation, 2% (wt/wt) glucose was added through the water. After the incubation phase, soils were removed from the cylinders and broken down by drop-shattering until they passed a 1 cm mesh. Then, they underwent a wet-sieving procedure on a 1000 and 250 µm mesh, respectively. The respective fractions were dried, and the weight determined. For further analysis, subsamples from the homogenized fractions were analysed by inductively coupled plasma mass spectrometry. The PET <63 µm incubation showed that 72.9 ± 7.2 % of the recovered MPs were detected in the fractions >250 µm. Using different MPs sizes of the same polymer (PET) will elucidate the effect of size on their incorporation into WSAs. Contrastingly, different MPs polymers of the same size range will shed light on the influence of the MPs chemical composition. Finally, testing two distinct soil textures with the same MPs polymer and size will lead to a better understanding of the importance of soil properties in this context.

3.23.A.T-05 Impact of Polyethylene Microplastics on the Vertical Migration of Pesticides in Soil
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With the revelation of microplastic contamination in soil, their interaction with organic chemicals has received increasing attention due to their hydrophobic surfaces, substantial sorption capacity, and large specific surface area. Our study aimed to extend the focus on single compounds to complex contamination by 20 pesticides which we applied to reference sandy soil. Stainless steel columns were filled with soil with or without the addition of 1% w/w polyethylene (PE) microplastics cryo-milled and sieved to a size of 200-600 µm of irregular shape. The columns were continuously rinsed with ten soil column pore volumes (PVs) of the pesticide-contaminated solution. The leachates were collected and measured every 0.2 PV using liquid chromatography-tandem mass spectrometry to derive breakthrough curves. The results showed that migration was positively correlated with hydrophobicity (as DOW and KOC), while the leaching order of multiple compounds was unaffected by PE microplastic contamination. However, PE microplastics promoted the vertical migration of pesticides in the soil, as previously reported in the literature. Overall, our results indicate that the sorption capacity of soils contaminated with microplastics for such chemicals may be decreased, enhancing the potential of groundwater contamination.

3.23.B Plastics in Terrestrial Ecosystems: Balancing Applications with Impacts, Fate and Hazards

3.23.B.T-01 Micro-Nanoscale Polystyrene and Polyvinyl Chloride Co-Exposure Impacts the Uptake and Translocation of Toxic Elements and Pesticides by Lettuce and Wheat

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Micro-nanoplastics (MNPs) are widespread emerging environmental and food contaminants. The ability of MNPs to influence the fate and effects of other environmental pollutants (EPs) in terrestrial environments and in edible plants is a topic of great concern. Investigations were conducted to evaluate if co-exposure to MNPs (polystyrene, PS; 20 or 1000 nm; or polyvinyl chloride, PVC, <10 µm, unaged or aged) had an impact on the accumulation of other EPs (arsenic, lead, chromium, boscalid, PFOS) in lettuce or wheat. Under hydroponic conditions, lettuce was co-exposed to PS at 10 or 50 mg/L, and to 1 mg/L of each EP. In a soil study with lettuce, the PS and EPs were at 50 and 10 mg/kg, respectively. Phytotoxicity was enhanced by PS under both growth conditions, particularly nanoscale PS (nPS), although impacts were less in soil-grown plants. Nanoscale PS had a greater impact than microscale PS (µPS) on As fate; the As translocation factor from roots to shoots was increased 3-fold in plants co-exposed to nPS (50 mg/L). PS dose and size had a variable impact on boscalid uptake and translocation. Fluorescent microscopy analysis of lettuce co-exposed to MNPs and EPs suggests that nPS enters the roots and translocates to the leaves, while µPS remains mostly in the roots. Pyrolysis-GC/MS showed that in soil, EPs significantly increased the translocation of nPS in lettuce shoots from 4.43 ± 0.53 to 46.6 ± 9.7 mg/kg, while the concentration of µPS in the shoots was unchanged with EPs. Separately, co-exposure to unaged PVC increased the root and shoot accumulation of As, Cr, and Pb by 3.1-, 2.1-, and 1.5-fold,

respectively; for aged PVC, the toxic element increases were 3.9-, 2.6-, and 2.1-fold, respectively. Soil-based experiments with wheat under similar co-exposure conditions are ongoing, as are molecular analyses to elucidate key mechanisms of action. These findings demonstrate that co-exposure of MNPs with other EPs can significantly impact co-contaminant accumulation and toxicity, presenting an unknown risk to humans and other receptors.

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3.23.B.T-02 Single- and Multigenerational Toxicity of Seven Mulching Film – Derived Microplastics in Single Species Tests with Nine Soil Invertebrates

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Agricultural plastics like mulching films and plastic fleece are beneficial for increasing yield while decreasing resources, causing a rise in their use over the past decades. However, their degradation and/or fragmentation causes microplastics (MPs) to accumulate in agricultural soils, where their effect on soil invertebrates remains largely unknown. Single species toxicity tests were therefore performed, using seven different MP materials derived from agricultural plastics and nine soil invertebrates: *Eisenia andrei*, *Enchytraeus crypticus*, *Ceratophysella denticulata*, *Folsomia candida*, *Heteromurus nitidus*, *Sinella curviseta*, *Porcellio scaber*, *Tenebrio molitor* and *Lasius niger*. Concentrations up to 5% (w/w dry soil) were included in all tests, with exposures done in or on Lufa 2.2 natural soil for all animals except *T. molitor*. Survival was assessed in all single species tests and different sublethal endpoints like reproduction, establishment of brood chambers or growth and moulting were included. Multigenerational exposures were performed using four of the nine animals and multiple MP materials. Generally, MPs did not affect the survival of the tested soil invertebrates in a dose-dependent manner, up to the highest tested concentration of 5% (w/w dry soil). Sublethal effects were also not always observed up to the highest test concentration, but they were observed for animal growth, oxidative stress induction in *E. andrei*, colony founding in *L. niger* and immune response in *P. scaber*. Besides the direct impact of MPs on soil invertebrates, physicochemical properties of the soil were also assessed. Multigenerational exposure did not increase effects compared to the single-generation exposures, but did show long-term effects on earthworm biomass at the highest exposure level in the 2nd generation. Profound changes in water holding capacity (WHC) and pH (0.01M CaCl₂) were observed, with some plastics causing a WHC increase of at least 50% and a soil pH increase of at least 1.2 pH units at 5% MPs (w/w dry soil). These changes in physicochemical properties may be indicative of soil structure and abiotic property changes in the soil, which may have an indirect impact on soil invertebrates and may impact soil communities over generations. Generally, annelids (*E. crypticus* and *E. andrei*) appeared slightly more sensitive towards MP exposures than the tested arthropods (*Collembola* and *P. scaber*); however no clear explanation for this can currently be provided.

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3.23.B.T-03 Microplastics Originated from Agricultural Mulching Films Affect Eechnytraeid Multigeneration Reproduction and Soil Properties

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Microplastics (MPs) are increasingly entering agricultural soils, often from the breakdown of agricultural plastics (e.g., mulching films). This study investigates the effects of six realistic MPs from different mulching films: two conventional polyethylene (LLPE-1 and LLPE-2) and two biodegradable (starch-blended polybutylene adipate co-terephthalate; PBAT-BD-1 and PBAT-BD-2), and two fabrics: ribbon fabric for direct cover (PPDE-black) and mulching fabric (PPDE-white). MPs were mixed into Lufa 2.2

soil at concentrations of 0.005%, 0.05%, 0.1%, 1%, and 5% (w/w dry soil) to reflect both realistic environmental levels and worst-case scenarios. Effects on *Enchytraeus crypticus* reproduction over two generations and six important soil properties were studied. PBAT-BD-MPs notably reduced enchytraeid reproduction in the F0 generation, with a maximum decrease of $35.5 \pm 9.6\%$ at 0.5% concentration. F1 generation was unaffected by PBAT-BD contamination. LLPE-MPs had a more substantial reproductive impact, with up to a $55.3 \pm 9.7\%$ decrease at 5% LLPE-1 concentration compared to the control, showing a dose-related effect except for 1%. PPDE-MPs impacted the enchytraeid reproduction significantly at the highest MP concentration, with a maximum reproduction decrease of 56.6% compared to the control. All MP types also significantly affected soil water holding capacity, pH, and total carbon. The most significant impact was recorded for PPDE-MPs, which increased the water-holding capacity by 71.8 and 52.7% compared to the control for PPDE-black and PPDE-white, respectively. PPDE-white also caused a significant increase in pH from 5.18 in the control to 7.41 at the 5% of MPs. These differences may explain the reduction of enchytraeid reproduction. On the other hand, although the MP addition of LLPE and PBAT-BD also significantly and dose-relatedly changed soil WHC_{max} and pH, these changes in soil properties were considered too small to affect enchytraeid reproduction substantially. The reproduction of enchytraeids was, in this case, probably influenced by the release of additives and/or small molecular compounds from MPs, having adverse effects. In summary, our results highlight the potential negative impacts of MPs originating from real agricultural plastics on soil health and raise concerns about the role of agricultural plastics in sustainable agriculture and food safety.

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3.23.B.T-04 Microplastic Effects on Soil Nematodes: Main Factors and Species-Specific Differences

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Microplastic (MP) is becoming an emerging threat to biodiversity and function of terrestrial ecosystem. Although soil is the primary sink for MP, the effect on the soil microfauna is not fully understood, especially in regard to the difference between biodegradable and non-biodegradable plastics.

The impact of MPs on the ecotoxicological model, the nematode *Caenorhabditis elegans* was investigated following a modified protocol of ISO 10872. Different fitness parameters including growth, fertility, and reproduction were evaluated. Nonbiodegradable low-density polyethylene (LDPE) and a biodegradable plastic mixture with 85% polylactic acid (PLA) and 15% polybutylene adipate-co-terephthalate (PBAT) were tested two size classes and four concentrations in three soil substrates (loamy sand, sand, loam). Also, effects of light and microbial degradation of MP were tested. The results show that toxicity varied with the MP concentration and soil types. Before aging, only LDPE inhibited the fitness of *C. elegans* while PLA/PBAT did not. Under aged conditions, both plastic types showed negative effects on nematodes, indicating that biodegradable MPs can become toxic after aging.

To investigate how different nematode species respond to MPs in a semi-natural soil environment, four bacterial-feeding nematodes, i.e., *Acrobeloides buetschlii*, *C. elegans*, *Cephalobus* sp., and *Diploscapter coronatus*, were tested in a modular system. The nematode behaviors of movement, food foraging and choice were tested, with LDPE and PLA/PBAT mixed with soil and loaded into the module according to the experiment setup. Both MP types reduced nematode movement, and had a potential to influence food foraging and choice in soil. These behavioral changes differed among nematode species. The difference between soil and agar were compared for food choice. The latter was related to MP amount and type, nematode species, and observation time, on agar plate, while in soil condition the impact on nematodes was minor.

Overall, the fitness of *C. elegans* can be negatively affected by MP, with impact differing due to soil and plastic types and concentrations. Moreover, species-specific response influences the impact of MP on nematode behavior in soil.

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3.23.B.T-05 Effects of Microplastics from Mulching Films on Soil Invertebrate Communities in Agricultural Field Plots from Three Different Geographical Regions

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The use of plastics has increased dramatically over the last decades, and so has emission to terrestrial ecosystems. In the environment, physical and chemical processes may cause fragmentation of plastic, leading to particles smaller than 5 mm, so called microplastics (MPs). A main pathway for MPs into soil is the use of plastic mulching films in agriculture. Knowing that MPs could pose a risk for soil biota, an increasing number of studies has recently assessed their toxicity to soil invertebrates, mainly in laboratory single-species toxicity tests, using unrealistic high concentrations. Such tests, however, cannot account for the complexity of interactions between MPs and soil organisms due to their controlled conditions and limited duration. Therefore, the main aim of this study was to unfold the long-term direct and indirect ecological effects of MPs on soil biota, under realistic environmental conditions.

Field-plot experiments were conducted at three sites representative of different agricultural and bioclimatic zones across Europe (Spain, Germany, Finland). Micronized pellets made from two recycled mulching films, conventional polyethylene (PE-MP), and biodegradable polybutylene adipate terephthalate-starch-blend (PBAT-BD-MP) were applied to the top 10 cm soil layer of 3.5 m x 4.5 m field plots and mixed with a rotavator to obtain nominal environmentally relevant concentrations of 0.005% and 0.05% (w/w). Each treatment had five replicates. Barley was sown on the fields for two consecutive years (2022, 2023). At the end of both growing seasons, the crops were harvested, and soil invertebrate samples were taken to assess the soil invertebrate community composition.

The number of earthworms per plot varied considerably between and within countries. In 2022, Spain suffered from severe drought and hardly any earthworms and no enchytraeids were found. In Germany and Finland, no differences in earthworm numbers were seen between treatments, but in Finland less juvenile earthworms were present at the high PE dose. In both countries, in 2022 enchytraeid numbers were lower at the high PE-level (0.05%), but only slightly decreased in other MP treatments. In 2023, no MP effects were found. In Spain, in 2023 enchytraeid numbers were reduced especially at the high BAT-MP and PE-MP concentrations. Microarthropod (springtails, mites) samples are still being analyzed, and results will be presented at the SETAC Europe meeting in Vienna.

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3.23.P Plastics in Terrestrial Ecosystems: Balancing Applications with Impacts, Fate and Hazards

3.23.P-Th315 The Impact of Microplastics on Nematode *Caenorhabditis elegans*

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Plastic waste has been found in the environment since the 1960s and even the most remote areas of the world have been polluted by it. While this problem is quite well described in Earth's oceans, other regions are just now starting to be investigated. As a result, micro- and nanoplastics (MNPs) are found in many more matrices, and soil is one of the largest global reservoirs of those particles. Yet research into the effect of MNPs on soil ecosystems is currently greatly insufficient. This work is part of the international PAPILLONS project Plastic in Agricultural Production: Impacts, Life-cycle and LONG-term Sustainability which plans to address this knowledge gap. Animals from phylum Nematoda are among the most numerous multicellular organisms on Earth, and their presence in soil is key to the biogeochemical cycles of several essential nutrients. That's why the soil reproductive test with nematode *Caenorhabditis elegans* was extensively optimized to culminate in the testing of four different kinds of plastics identified as most frequently used in European agricultural mulch films.

Two conventional materials consisting of polyethylene (PE-1 and 2) and two biodegradable ones made of polybutylene adipate terephthalate (PBAT-BD-1 and 2) were tested in standard soil LUFA 2.2 in

concentrations 0; 0.005; 0.05; 0.1; 0.5; 1 and 5% (w/w) of particles per 0.5 g of soil, the average size of these particles being 24,7 107,8 µm. The results indicated that with an increased amount of PE-1, the average reproduction of nematodes decreased up to the lowest number of juveniles at 5% of MNPs added. Conversely, for PBAT-BD-2, the reproduction was highest at concentrations of 1 and 5%. During literary research, a significant data gap was identified on the effect of microplastics on the nematode *C. elegans* in soils. To assess the potential impact of using conventional and biodegradable plastics in agriculture on these and other organisms, determine the associated risks, and take the necessary protective measures, having enough relevant information is vital. Research focused on the impact of agriculture on soil ecosystems represents one of the steps we need to take to ensure the proper function and fertility of soils for future generations.

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3.23.P-Th316 Behavioural Response of Terrestrial Crustaceans to Agricultural Microplastics

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Agriculture is one of the most important sources of microplastics (MPs) in soil. Recent studies have shown that the effects of MPs on the survival and reproduction of terrestrial invertebrates are not severe at environmentally relevant concentrations. However, it is unclear whether this also holds for their behaviour. The avoidance behaviour of organisms is considered an environmentally relevant endpoint, as their habitat function is reduced when they avoid contaminated substrates. On the other hand, preference for a contaminated environment increases their exposure to pollutants.

We investigated the behavioural response of terrestrial crustacean woodlice *Porcellio scaber* to MPs derived from two agricultural plastics: black polypropylene ribbon fabric for direct cover to suppress weed growth, and white mulch fleece for frost protection. We performed a 48-hour soil selection test and a 3-hour video recording test. In the 48-hour soil selection test, the animals chose between control and MP-polluted soils, which were housed in two identical test containers connected by a tunnel. The animals were exposed individually and in groups of ten, and their behaviour was assessed as the percentage of animals on the control versus MP-contaminated soil. In the second exposure scenario, individual animals were recorded for 3 hours in transparent pots divided into two equally sized chambers by a barrier with a passage in the centre. The proportion of time on control compared to MP-polluted soil and the activity of the animals on each soil type (locomotion time, animal speed and total path length) were tracked. In both scenarios, the following pairs of exposure concentrations were tested: 0-0, 0 0.05%, 0 0.5% and 0 5% (w/w) MP for 48-hour exposure with additional 0-1% MP for video tracking. In most treatments, the woodlice did not discriminate between the control and the MP-contaminated soils. However, a clear preference for the soil spiked with 5% MPs from the black direct cover was found in the individual exposure. Video recordings of individual isopods confirmed the preference of soil with 5% of this MP, they spent a longer time, and their speed of locomotion decreased while time of locomotion increased on that soil. When exposed to the mulching fabric fleece MPs no soil discrimination was noticed. In conclusion, our results show that some agricultural MPs can influence the behaviour of woodlice.

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3.23.P-Th317 Soil Litter Feeders Impacted by Low Concentrations of Microplastics. The Case of Earthworms, Ants and Slugs in the MINAGRIS PROJECT

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Plastic pollution is one of the international problems that is facing our modern society. Macro, meso and

microplastics are found in different types of terrestrial environments, from agricultural soils to parks, or conservation areas. In the EU MINAGRIS project, we try to assess and understand the interaction between plastic particles with different stressors at different soil conditions. Therefore in WP4, one of the tasks was consecrated to the assessment of the impact of microplastics on soil fauna. The main objective of this task was to assess mortality, growth rate and behavior of different soil invertebrates. At laboratory, with control conditions of temperature and moisture, experiments were performed with 3 main types of soil organisms: earthworms (anecic, epigeic and endogeic), ants and slugs; 3 types of soils were used (sandy, sandy loam and clay soils) and 3 types of plastics (starch based, PBAT and polyethylene) were implemented with very low microplastic concentration doses (0.01% and 0.1%). Finally the main results reveal that biodegradable plastics together with pesticides might impact anecic earthworm growth rate significantly at 7 days, compared with other type of plastics. At the long term (60 days), anecic earthworms seem also be impacted with fossil-based biodegradable plastics (PBAT). Ants' behavior are perturbed with the presence of plastic particles, separating the plastic particles from the rest of the food materials. Slugs, like litter feeders lose weight with leaves mixed with low concentrations of microplastics. No significant differences were observed between endogeic earthworms with low concentration of microplastics. Preliminary results indicated that low concentrations of microplastics also impact soil fauna. More data analysis is needed for connecting the impact of low concentration of microplastics on soil ecosystem services provided by soil fauna.

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3.23.P-Th319 Microplastics Derived from Agricultural Mulch Films Indirectly Affect Terrestrial Organisms and Microbial Activity Due to Altered Soil Properties

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Microplastics (MPs) in soil bind minerals and organic matter, integrating into the soil matrix. This incorporation can alter soil physical properties, such as bulk density, aggregate stability, and water-holding capacity. Our previous results showed alterations in soil physical parameters due to the presence of MPs derived from agricultural mulch films. Given that altered soil properties could have impacts on soil biota, our present research investigates if selected MPs affect terrestrial organisms and soil microbial activity. We exposed terrestrial crustacean *Porcellionides pruinosus* (Crustacea, Isopoda) to soil with three types of MPs (LDPE, PBAT, starch-based PBAT) at 1%, w/w concentration in sandy loam soil for 14 days. Pots used in the experiment were filled with dried and sieved with sandy-loam soil and mixed with MP. Pots were placed in randomized order in specially designed pools with gravel and jute at the bottom to facilitate wetting and buried in soil. After wetting organisms were introduced to the pots for a 14-day exposure period.

After the experiment, we analysed the survival of the test organisms, the level of selected immune parameters (total haemocyte count, differential haemocyte count, haemocyte viability and phenoloxidase-like activity), the activities of two stress-related enzymes (AChE: acetylcholinesterase and GST: glutathione S-transferase) and the metabolic activity (electron transfer system activity, ETS). In addition, the microbial activity i.e. CO₂ production in the soil samples taken from the control and the soil treated with MPs was analysed.

The survival of the test animals was not affected by the exposure to three different types of microplastics in the soil compared to the control group. We also did not observe any changes in enzyme activities for all tested MPs. However, we observed a significant decrease in the total haemocyte and phenoloxidase-like activity in *P. pruinosus*, after exposure to 1% STARCH in soil. These results coincide with increased microbial activity in the soil with added PABT MPs and especially Starch based MPs. We speculate that the observed alterations at the immune system level of a *P. pruinosus* are due to the indirect effects of MP exposure. This implies that selected terrestrial organisms could be employed to measure the indirect effects of plastic pollution, which is of significant importance for properly assessing the impact of plastics on the environment.

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3.23.P-Th320 Sublethal Effects of Polypropylene Agricultural Microplastics on *Eisenia Andrei*
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We examined the avoidance behavior of the earthworm *Eisenia fetida* when exposed to agricultural plastics, specifically two types P8 (polypropylene [PP] ribbon fabric for direct cover) and P9 (PP mulching fabric), under controlled conditions. With the rapid increase in plastic use since the 1950s, over 300 million tons of plastics are produced annually. Plastics, composed of long-chain organic polymers, degrade slowly in the environment into microplastics (<5 mm), posing risks to ecosystems. In agriculture, plastics are extensively used for mulching, packaging, and irrigation, leading to their accumulation in soils. Microplastics in agricultural soils can persist for centuries, raising concerns about their ecological impacts.

The experiment was conducted using ISO 17512-1:2008(E) standard. Soils mixed with microplastics at concentrations of 0.05%, 0.5%, and 5% were placed in aluminum trays, with clean soil on the opposite side. Worms were introduced at the center and allowed to choose between the sides. After 48 hours, the distribution of worms was counted to assess avoidance behavior.

Results showed that at concentrations (0.05 0.5%) of P8, no significant avoidance was observed. However, at 5%, over 90% of worms preferred the clean soil. For P9, avoidance was evident even at 0.5%, with over 75% of worms choosing clean soil, and at 5%, over 90% displayed avoidance. These findings indicate that earthworms are more sensitive to certain types of plastics and respond strongly to higher concentrations of microplastics.

The avoidance behavior of earthworms has significant ecological implications. Earthworms play critical roles in maintaining soil health by enhancing organic matter decomposition, nutrient cycling, and soil structure. Their displacement from contaminated soils could disrupt these ecosystem services, potentially degrading soil quality. While average microplastics concentrations in agricultural soils are typically lower than the highest tested levels, some hotspots may reach or exceed such concentrations.

The study underscores the risks posed by the increasing use of agricultural plastics. The variability in earthworm responses to different plastic types suggests that the diversity of plastics used in agriculture could result in varying impacts on soil organisms. As the issue is cumulative, it highlights the need for further research and sustainable practices to mitigate the long-term effects of microplastics on agricultural ecosystems.

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3.23.P-Th321 First Assessment of the Impact of Plastics on Soil Communities: A Case Study in France

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Plastics presence and degradation in soils constitutes a growing environmental concern, with significant implications for soil health and biodiversity. Despite extensive research on plastic pollution in marine and freshwater environments, there is an urgent need to improve our understanding of the impacts of plastic debris in terrestrial habitats, where microplastics and nanoplastics can be found in greater quantities than in aquatic ecosystems. Agricultural soils are of particular concern, as they are end-receivers of several sources of plastics, such as compost from household waste or sewage sludge. Furthermore, these soils which cover about 25% of Europe's soil surfaces are of crucial importance for global food production, and provide support for key ecological processes such as carbon storage and nutrient cycling.

The present work was performed in an agricultural field which received compost from household wastes; and the composition of communities of ground beetles, spiders and plants was examined.

The study site is located in France and comprised two adjacent plots in fallow: one plot was contaminated with household waste compost enriched in plastic particles, while the second plot free of compost serves as control. We used pitfall traps to assess the activity density of ground beetles and spiders. We also used

vegetation quadrats to assess plant cover. Diversity indices were then calculated on the data of species collected.

Species richness and Shannon's diversity were significantly higher in the contaminated plot compared to the control for the communities of spiders, ground beetles and plants. Also, Pielou's evenness was significantly higher in the contaminated plot compared to the control for the communities of plants. These results suggest that plastics in the soil could affect - directly or indirectly - the composition of spiders, ground beetles and plants communities, and the dominance of certain plant species. Additionally, we examined the functional diversity of the spider communities, and the presence of plastics did not affect spiders' community weight means for size or hunting strategy.

Our study provides rare and novel field data on soil plastic contamination and its impact on aboveground communities, as it is the first study of this kind to our knowledge. It sheds new light on the impact of plastic waste on soil ecosystems, and it could be a starting point for a deeper understanding of the response of communities to plastic pollution.

3.23.P-Th322 Degradation Behaviour of a Seed Coating in an OECD TG 307 Simulation Test and Quantification with Pyrolysis-GC/MS and FFF-MALS

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The fate of polymers is in the focus of the new restriction (Commission Regulation (EU) 2023/2055) issued by the European Commission on microplastics intentionally added to products used for specific purposes. The criteria for restriction are based on the biodegradability of polymers, which is determined according to OECD test guidelines (TG), for example TG 301 and TG 307. Only synthetic polymers are considered in the restriction. Therefore, producers strive to shift to natural polymers, which are considered to be biodegradable by default, and synthetic polymers which show proof of fast biodegradation (i.e. >60% mineralisation within 28 days, measured in TG 301). The degradation rate and formed degradation products of respective polymers are often unknown. While natural polymers are considered degradable by default this does not mean that they would pass those degradation tests. Also, in our project an OECD TG 301 test has shown that combining different natural polymers in a seed coating resulted in less than 60% mineralisation in 28 days. In order to address the biodegradability of the seed coating a simulation test (OECD TG 307) was chosen as it would be foreseen in the restriction. For this purpose, the main polymer sodium alginate was radiolabelled with ¹⁴C-Bromethanol and the seed coating was produced with the ¹⁴C-radiolabelled material. The ¹⁴C-radiolabel allows to follow degradation processes in the complex soil matrix and to determine mineralization (CO₂ formation) even at environmental relevant polymer concentrations. Further, a density separation was developed to quantify the degradation of the polymeric seed coating as well as measure a shift in the molar mass. During specific time intervals the seed coating is separated and analysed with pyrolysis-gas chromatography and mass spectrometry (GC/MS) and field-flow fractionation coupled to a multi-angle light scattering detector (FFF-MALS) and radio-detection. As the seed coating degraded using oxidative extraction methods, a density separation was used. Often zinc chloride solution is utilized for microplastic density separation but we observed degradation of the seed coating using this salt. Instead, sodium iodide was chosen for density solution reaching densities up to 1.9 g/cm³. A separation recovery of 87.8 ± 5.3 % was achieved with this method. Details of the OECD TG 307 test, analysis of the polymer concentration with pyrolysis-GC/MS, and the molar mass shift with FFF-MALS will be presented.

3.23.P-Th323 Microplastic Generation and Changes in Functional Chemistry Under the Combined Effects of UV and Thermal Oxidation with Abrasion

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This study compares the generation of microplastics (MPs) from plastics oxidized by thermal and ultraviolet (UV) radiation processes. Our group has found that open-pit waste burning in underserved areas contributes to MPs generation showing signs of thermal and UV oxidation. However, the mechanisms behind the MP generation under these two oxidation processes are not fully understood. The plastics selected in this study include low density polyethylene (LDPE), polypropylene (PP), and polystyrene (PS), and were all obtained as consumer products. To understand changes in functional

chemistry, MP generation and reactivity in aqueous media, thermally, ultraviolet (UV), and non-oxidized plastics were subjected to mechanical abrasion conditions that promote the fragmentation of plastic pieces into MPs. We used Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy to analyze the functional group and structural changes in the plastics before and after oxidation and before and after abrasion. Microplastic generation was quantified in terms of particle count using fluorescence microscopy, and in terms of weight with pyrolysis gas chromatography/mass spectrometry (Py-GC-MS). Results show that UV and thermal oxidation increase carbonyl (C=O) and ester (C-O) functional groups, evidenced by higher carbonyl index (CI) values. Abrasion further modifies the chemical structure, introducing reactive oxidative sites due to polymer chain scission, as reflected by distinct changes in ATR-FTIR spectra. MP generation was highest in UV-treated plastics subjected to abrasion, with LDPE and PP showing significant weight loss and fragmentation. In contrast, PS exhibited resistance to abrasion post-oxidation, likely due to alterations in its glass transition temperature (T_g). These findings highlight the combined impact of oxidation and mechanical abrasion on plastic degradation. The chemical changes observed may influence the environmental behavior, toxicity, and interactions of MPs, particularly in ecosystems affected by open burning and unmanaged plastic waste.

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3.23.P-Th324 Optimizing Microplastic Monitoring Techniques Using an Experimental Mass Based Approach

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Microplastics are a diverse suite of ubiquitous contaminants ranging from 1 micron to 5 millimeters that include a wide array of morphological features. Developing a standard operating procedure to collect microplastics during stormflow conditions is essential to harmonize data across regions and waterways. There is currently no standard protocol for collection and analysis of microplastics, making comparisons between studies difficult to assess. This study aims to develop a standard for microplastic collection in river stormflow for the state of California using a flume and environmental river samples. Multiple sampling techniques, from simple surface grab to sophisticated suspended sediment net sampling, were compared using four polymer types with different densities in an experimental flume using a mass based analysis approach. Various depths, velocities, and sampling orientations were tested and analyzed to compare sampler performance under several hydrologic conditions. We hypothesize that depth integrated isokinetic approaches will result in the most representative sample across the flow field, as surface and mid depth samples will not capture all transport modes. In addition to the experimental work, we used these same sampling methods in Los Angeles County rivers to compare with environmental data in urban systems. This standard will assist with consistent data across studies and allow for impactful comparisons globally, generate a better understanding of microplastic fate and transport, and produce more accurate flux models from coastal watersheds to the marine environment. Our recommendations will be used by state municipalities and researchers to monitor microplastics across California.

3.23.P-Th325 Improving Controlled Separation of HDPE and PP via Electron Beam Technology for Detection of Microplastics

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Although microplastic pollution is a global environmental issue, analysis remains a challenge. While most established methods, such as Raman microscopy and TED-GC-MS, are very time-consuming and costly, differential scanning calorimetry (DSC), after prior enrichment by electrostatic separation, requires less time and investment and is therefore considered a routine-capable method. Application of electrostatic separation and DSC enables Microplastic-Analysis in particular matrices. A DSC measurement investigates the phase transitions in semicrystalline polymers as they are heated or cooled. During melting or crystallization, a polymer-specific signal is generated, allowing the identification and quantification of

an unknown polymer sample. However, the signals of some critical polymer pairings, such as HDPE and PP, overlap when both are present simultaneously, making their parallel identification and quantification challenging.

To address this issue, the effect of electron beam treatment on the physicochemical properties of these polymers and their controlled fractionation in electrostatic separation was investigated. The results demonstrated that a controlled fractionation of HDPE and PP in a corona roller separator is possible after electron beam pretreatment. The absorbed primary charges from the initial electron beam treatment induce distinct charging properties which persist for at least two days (the maximum duration studied), that influence the behavior of polymer particles during electrostatic separation. Under optimal irradiation and storage conditions, a controlled separation of HDPE and PP particles is feasible, enabling the quantification of polymers using DSC. Challenges remain in accounting for the influence of electron beam treatment on the DSC signal, scaling up the particle mass in the separation process. Additionally, further investigation is needed to determine whether the effects of electron beam treatment are applicable to other critical polymer pairings, such as PET/PVC and PET/PA6.

The findings provide valuable insights into the differential enrichment of HDPE and PP and are relevant not only to the analysis of microplastics but also to the optimization of plastic recycling, particularly regarding the pretreatment of plastic waste with electron beam radiation.

3.23.P-Th326 Abundance and Characteristics of Anthropogenic Litter Along the Tanzanian Shores of the African Great Lakes: Volunteer Involvement, Outreach and Stakeholder Engagement

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Environmental pollution due to mismanaged anthropogenic litter is one of the most pressing issues facing the world today. To reduce existing environmental burdens of litter, clean-up events have increased in popularity. Here, we report on the results of the Clean Shores, Great Lakes project, in which we conducted 69 volunteer-based clean-ups along the Tanzanian shorelines of the African Great Lakes (Victoria, Tanganyika, and Nyasa [also known as Lake Malawi]). A total of 5483 volunteers engaged with the project across all clean-up sites, collecting 431,328 pieces (25981 kgs) of anthropogenic litter. Expressing the data as normalized to area (items m⁻²) or effort (items individual⁻¹ hour⁻¹) revealed that the interpretation of a polluted site varies with units. Litter abundance varied from 0.06 to 13.89 items m⁻² and 5.2-706 items individual⁻¹ hour⁻¹. Plastics were the major litter type constituting 75% of all litter (mean = 74.2 ± 12.5 % across all sites), while clothing (10.9 ± 9.5 %) and fishing gear (3.7 ± 5.2%) were also prevalent. Two items constituted > 40 % of all collected litter, namely plastic beverage bottles (average 23.7 ± 17.1 %, range 0-72.9 %) and plastic bags (average 19.7 ± 13.2 %, range 1-50.3 %). Prioritizing intervention measures on these two items could reduce litter leakage significantly. Further, a holistic approach was undertaken through voluntary engagement, citizen science involvement, stakeholder dissemination and school visits to increase public awareness. Clean-up data when disseminated to relevant policymakers and stakeholders can motivate activity for the environmental management of anthropogenic litter and plastic pollution.

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3.23.P-Th327 Reducing Microplastic Emissions from Aquaculture Through Optimisation of Infrastructure and Cleaning Technologies

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The aquaculture industry is dependent upon large amounts of plastic infrastructure, which can contribute to the direct release of microplastic (MP) into the marine environment. Net cleaning procedures can cause net abrasion, potentially leading to coating particles and polymer fibres being released. Here, we apply safer and sustainable by design principles to quantify the reduction of MP emissions by introducing feasible reduction measures through the selection of specific combinations of net polymers, coatings and cleaning technologies. A laboratory experiment simulating in situ mechanical abrasion was performed to quantify

and compare MP release from different nets. The tested net types included new Nylon, HDPE, Dyneema, and Entex net, used nylon net, as well as two coating types, standard and premium, used to extend net life. The collected particulate material was quantified and characterised using gravimetry, microscope imaging and pyrolysis GC-MS. Nylon nets release higher quantities of MP compared to HDPE, Dyneema and Entex, while coatings interacted differently with the various net materials. There was no change in MP release from coated Dyneema nets, but an increased particle release from the coated nylon nets. For nylon nets, used materials released more MP than new ones, with used nylon net samples from different depths exhibiting different particle release profiles. The results indicate reduced MP emissions from PE based nets compared to nylon. However, it is important to note that nylon nets can be chemically recycled, while this is not currently possible for PE nets (although testing for mechanical recycling is ongoing). In a field study, a partial aquaculture pen net comprised of used nylon net and coated new nylon net panels was constructed and deployed at sea. Each type of net material was subjected to 3 different washing techniques simulated approx. 10 months at sea: (i) a standard high pressure cleaner (Stealth Cleaner), (ii) a cavitation cleaner (Meox cleaner), and a brush-based grooming ROV (Watbots ROV). Water samples (min 200 L per replica) were pumped through a stack of large surface stainless steel filters with mesh sizes of 300 and 10 μm . The samples are currently being processed and analysed by imaging μFTIR to quantify and characterise the MP emissions from each combination of net type and cleaning technology. Preliminary results indicate a predominance of nylon MPs, but a broad range of other polymer types are also present.

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3.23.P-Th328 Assessing the Microplastic Contribution from Sewage Sludge to Agricultural Soils with Regular Sludge Application

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The transition to sustainable agriculture emphasises the adoption of organic alternatives, such as biosolids, in place of inorganic fertilisers that contribute to the circular economy. Biosolids are widely utilised in the United Kingdom as a fertiliser for replenishing organic matter in agricultural soils. However, their application has been identified as a potentially significant pathway for microplastic (MP) contamination in terrestrial environments. This study aims to evaluate the presence and concentration of microplastics in soil obtained from farmland soils in Southwest England, which have records of biosolid application history. Soil from agricultural land that has not had biosolid for a prolonged time, but has an otherwise similar land use history was used as controls for identifying non-sludge-derived microplastics. The collected soil samples were digested using Fenton's reagent, and a series of density separations using saturated ZnCl_2 were carried out to extract microplastics from the soil matrix. The biosolid amended soil accounted for 80-95% of the MP fibres and 6-20% of the MP fragments recovered. The average concentration of microplastics was 4,465 particles per kilogram of dry weight of soil, although there was significant variability across different fields, with values ranging from 1,200 to 11,400 particles per kilogram of dry weight. This variation highlights the complexity of microplastic contamination in soils affected by biosolid application. Additionally, soil samples were analysed for other properties such as water-holding capacity, pH, organic matter, NH_3 , NO_3^- , NO_2^- , and PO_4^{3-} . Organic matter was measured using the loss on ignition method, and nutrient analysis was performed using a Skalar Bluvision discrete analyser. The average organic matter, NH_3 , NO_2^- and NO_3^- content for biosolid applied fields were $10.38 \pm 1.18\%$, $1.11 \pm 0.48 \text{ mg/kg}$, $0.05 \pm 0.02 \text{ mg/kg}$, and $48.57 \pm 22.30 \text{ mg/kg}$ and for control fields were $11.83 \pm 1.32\%$, $1.45 \pm 0.06 \text{ mg/kg}$, $0.08 \pm 0.07 \text{ mg/kg}$, and $36.60 \pm 15.47 \text{ mg/kg}$, respectively. The higher concentrations of fibres in soil primarily come from synthetic textiles in sewage, and their tendency to entangle in soil aggregates contributes to their persistence. Aside from biosolid application, soil properties varied across the fields due to factors such as soil type, complexity of the matrix, geographical distribution, and land use.

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3.23.P-Th329 Ageing Conditions Alter the Effects of Leachates from Tire Wear Particles on Soil
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Tire wear particles (TWP) are one of the most abundant types of microplastics, and have emerged as a main contributor of microplastic pollution. Given the fact that tires are a complex mixture of rubber and chemical additives, previous research on assessing the effects of TWP has been mainly based on the leached fractions. Although physicochemical properties of TWP (e.g., chemical constituents or surface properties) and environmental conditions (e.g., UV, pH, temperature and salinity) are well known as main factor influencing the chemical leaching processes, there is a knowledge gap regarding different ageing conditions due to the lack of empirical data. In this study, we established a soil laboratory experiment using TWP leachates with three different ageing treatments (mechanical-, thermal-, and UV-ageing), and measured nine soil parameters (soil microbial activities and physicochemical properties) to investigate how ageing conditions influence the effects of TWP leachates on soil. We found that TWP leachates of different ageing treatments induced distinct effects compared to leachates from pristine TWP, and each ageing treatment showed different response patterns in different soil parameters. Compared to the non-aged treatment, the leachates of the mechanical-ageing treatments affected soil C/N ratio and α -1,4-N-acetyl-glucosaminidase activity, while the leachates of thermal-ageing treatment influenced soil litter decomposition, water stable aggregates, and pH. Our study provides solid evidence that the ageing conditions can alter the effects of TWP leachates on soil. This has further refined our knowledge of the ecological impacts of TWP, and highlighted the necessity of considering ageing condition as a crucial factor investigating the environmental effects of TWP.

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3.23.P-Th330 Is “Compostable” also “Eco-Friendly”? – The Fate and Effects of Compostable Plastic Bags After Composting

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Compostable plastic products are commonly perceived as an environmentally friendly alternative to conventional plastics. However, European standard tests to certify a material as compostable do not reflect realistic conditions in municipal composting facilities. Studies have shown that high numbers of microplastics derived from compostable plastic products remain in the compost and may have a higher resistance to enzymatic degradation than previously assumed. The compost containing the residual biodegradable plastic fragments is then distributed to agricultural fields. It has also been shown that compostable plastic products can contain chemicals that leach from the product over time. Negative effects of these leachates have been reported for aquatic organisms, but studies concerning the effects on soil systems are lacking.

In this recently started project we aim to determine if residual microplastics derived from already composted compostable plastic bags will degrade under field conditions within two years. For this we will characterise and quantify the biodegradable plastic fragments in the compost from a Dutch municipal composting plant. The characterised compost will then be placed in mesocosms on a Dutch field and sampled at the beginning of the experiment and after 3, 6, 12, 18 and 24 months. These samples will be analysed via Fourier Transform Infrared (μ -FTIR) spectroscopy for a particle based monitoring as well as NMR (Nuclear Magnetic Resonance) spectroscopy for a mass-based monitoring, allowing comparisons of mass and particle reduction.

Further we will assess how the chemical leachate and fragments from compostable plastic products affect earthworm fitness, reproduction and development.

By May 2025 we will be able to present our methodologies, and the first results of the degradation process after 3 months and the results of the earthworm reproduction tests.

As the use of compostable plastic products is expected to increase significantly in the coming years, we consider it essential to close the knowledge gap on the fate and effects of compostable plastic particles after they have undergone the conventional composting process and end up in agricultural fields. This

study would then be one step towards determining if compostable plastics are indeed environmentally friendly and in compliance with the European Green Deal, the Zero Pollution Action Plan and the European Soil Strategy.

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3.23.P-Th331 Towards Implementation of an Environmental Risk Assessment Framework for Microplastics in the Dutch Policy Context

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Microplastics are ubiquitous in the environment, including in soils, and pose a hazard to organisms. Environmental risks of microplastics remain uncertain, especially for soil ecosystems. In part, this uncertainty relates to the availability of reliable and relevant data and the extent to which risk assessment approaches can deal with the complexity of microplastics pollution. Environmental risk assessments (ERA) are at the basis of policy on chemical pollution. It is therefore important that an ERA framework for microplastics meets policy makers' needs for policy on microplastics and that it is supported by the scientific community. In this study we take an ERA approach developed by researchers from the Wageningen University (the Netherlands) and, through a series of workshop and a questionnaire, assess whether the approach fits the needs of Dutch policy makers and whether it is supported by members of the scientific community. The original focus of the study lies with the soil system. However, the consulted experts and policy makers were from various backgrounds and so the study outcomes are likely relevant to multiple compartments/policy domains. Among Dutch policy makers from national government we identified a clear need for an ERA of microplastics, although underlying reasons varied and included, among others, the setting of environmental quality standards, creating support for microplastics policies and identifying hotspots of pollution. The policy makers stressed the importance for providing perspectives for policy actions. A questionnaire targeted at scientific experts on the topic of ERA of microplastics showed that generally, there was broad support for use of the approach developed by the researchers from Wageningen University for (soil) microplastics policies. However, there are different perspectives on attributes of ERA of microplastics, for example, on the importance of including chemical effects and the specific needs for further development of the approach. Additional scientific perspectives on the topic will be collected via an online workshop. The findings of this study will lead to a better understanding of perspectives on ERA, how it meets the needs of Dutch policy makers, and specifically what else needs development or studies. Based on the input collected we will formulate and present recommendations to further science-based environmental policy on microplastics.

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3.23.P-Th332 Ecological Risk Assessment Framework for Microplastics in Agricultural Soils Amended with Biosolids

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This study conceptualizes a framework for ecological risk assessment of microplastics (MPs) in agricultural soils amended with biosolids. MPs in biosolids pose risks to soil biota, affecting soil health. The study highlights the complexity of assessing MP risks, considering not only MPs abundance, but also properties such as size, shape, and type. To develop this framework for ecological risk assessment of MPs in agricultural soils amended with biosolids, a literature review was conducted to systematically assess effects of different MPs properties on soil organisms. Earthworms, springtails, and the microbiome were considered as receptors. The study highlights the importance of understanding MPs fate in soil, since effects on soil biota can be time dependent. Furthermore, results show that organisms respond differently to similar MPs properties, increasing the complexity of assessing MPs risks in terrestrial ecosystems. This complexity also relates to MPs effects on soil properties, and indirect effects on soil biota. Further research is needed to address knowledge gaps for effects of specific MPs properties to better assess and manage ecological risks in agricultural systems.

3.23.P-Th333 Investigating Phytoremediation Potential via Retention Modules for Microplastic Contamination in Soils

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Microplastics (MPs) enter the environment through manifold direct and diffuse pathways, posing risks to flora, fauna, humans and entire ecosystems. One of the major sinks for MPs are soils. The common research opinion is that MPs, once reached into the soil, cannot be extracted anymore. Therefore, we want to investigate the chances and limitations of phytoremediation further. So far, there are only a few studies on phytoremediation in terrestrial systems, mostly limited to laboratory experiments. Our goal is to develop wooden modules, designed to retain MPs in floodplains as their soils are known to be highly polluted with MPs possibly entering the ground- and drinking water.

As preliminary studies, we mapped MP contamination of the Elbe river floodplains along several transects down to 30 cm depth to identify small-scale MP hotspots. Additionally, we investigated the intake capacity of plant roots by a 3-month laboratory experiment with osiers [*Salix viminalis*], planted in sand which we spiked with inhouse-MP-reference-particles. Per planting pot, we used 0.75 mg of MP in 2 kg sand; either LDPE or PA with particle sizes of 500-600 μm or 650-1050 μm , respectively. As a proof of concept, we built wooden frames (30x30x10 cm), filled up with sand or loam. In each of these retention modules, we planted common osiers [*Salix viminalis*] or purple osiers [*Salix purpurea*] in four different plant cover densities (w/out, 16, 36, 46 cuttings). Half of the modules with 36 osier cuttings got additionally interspersed with reed canary grass [*Phalaris arundinacea*]. All of the modules are going to be flushed three times with 10 litres of water, containing 1 g inhouse-MP-reference-particles per 10 litres (0.5 g LDPE + 0.5 g PA; 500 600 μm). Thereby, we want to test the differences in MP retention capacity depending on different grain sizes, plant cover densities and plant morphologies. We extract MPs with sodium metatungstate (1.8 g/cm³) or sodium chloride (1.1 g/cm³) solutions and analyse MPs with differential scanning calorimetry (DSC).

The first results of this comprehensive study will be presented at the SETAC exclusively. At the same time, we want to use the poster presentation to discuss other methods besides phytoremediation to remove MPs from soils.

3.23.P-Th334 Understanding Plasticizers in German Soil

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In the modern world plasticized PVC products can be found everywhere. Consequently, plasticizers are ubiquitous in the environment. Nowadays, the legacy plasticizers Di(2-ethylhexyl) phthalate (DEHP) and some short-chain phthalates are banned in the EU due to toxicological concerns. As a consequence, the industry underwent a shift in production towards high-molecular-weight phthalates and non-phthalates. However, there is a lack of data regarding environmental contamination, particularly in soil.

The objective of this research is to elucidate the prevalence of both, legacy and emerging plasticizers in German soil. This offers a more comprehensive understanding of the pollution than would be possible by focusing solely on phthalates, as has been the case in many previous publications. Furthermore, it will allow to monitor the potential environmental impact of substitute products, a field in which there are currently very few studies.

Samples were obtained from the German Environmental Specimen Bank (ESB), taken by its archive program since 2002 every four years from each of the three topsoil layers. The samples represent predominant ecosystems, with consideration of the varying intensity of anthropogenic influence and land use.

We present a modified approach for analyzing soil samples, which is adapted from our research group's previous work on plasticizers in different environmental samples. This is especially demanding since the purification and avoidance of contamination and high blank values represent a significant challenge due to the omnipresence of plasticizers, even in laboratory settings.

Based on the restriction and the resulting substitution of banned phthalates, it would be expected that their concentration in German soils has decreased in recent years. This could be found in previous studies by analyzing freshwater samples. However, this clear trend could not so far be confirmed in soil samples analyzed from 2002 up to 2018. Those phthalates, which have been banned in consumer products since 1999, still account for 40 to 90% of the total plasticizer contamination found in the soil samples.

Overall, the data obtained from this project may facilitate the formulation of conclusions regarding the deposition and distribution of plasticizers. Additionally, it may provide information on the efficacy of regulations, which appeared to be not fully sufficient.

3.23.P-Th335 Interactions between Microplastics, Heavy Metal Pollution and Antibiotic Resistant *Pseudomonas aeruginosa* in Sewage Sludge: Impacts on the Safety of Agricultural Soils

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Sewage sludge is a major by-product of wastewater treatment, with approximately 45 million dry tonnes produced annually worldwide from municipal wastewater treatment plants (WWTPs). Sludge from these WWTPs often contains microplastics (MPs), heavy metals, and pathogens, presenting environmental and health risks if applied as fertilizer. MPs in the sludge can act as carriers for heavy metals and encourage the colonization of antibiotic-resistant bacteria like *Pseudomonas aeruginosa*.

An analysis of sludge samples from eight WWTPs in Hungary, using two common analytical techniques Fourier-transform infrared spectroscopy and pyrolysis gas chromatography-mass spectrometry revealed an average MP concentration of 60.33 particles/g (dry weight) and 75.46 µg/g (dry weight) by mass. The most frequently identified polymers were polyethylene, polyester, and polypropylene, known for their high metal adsorption properties. Among the heavy metals, zinc was the most abundant, with an average concentration of 628.86 mg/kg, although all levels were within EU regulatory limits.

P. aeruginosa strains isolated from the sludge showed biofilm formation abilities, heavy metal tolerance, and antibiotic resistance, associated with specific genetic markers identified through whole-genome sequencing. These findings suggest that MPs from sludge may act as vectors for both heavy metals and antibiotic-resistant bacteria in soil, raising significant risks for agricultural and ecological health when sludge is used as fertilizer.

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3.23.P-Th336 Investigating the Impacts of Extended Leaching from Virgin and UV-Aged Conventional and Compostable Plastics on Freshwater Organisms: A Focus on Inorganic Additives

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The mobility of chemicals associated with plastics, including additives and contaminants, raises serious ecological concerns for terrestrial ecosystems. Under specific conditions, particularly those enhanced by plastic aging, these chemicals can leach into the surrounding environment, leaving them increasingly exposed. Although the toxicity of individual hazardous chemicals is documented, the combined toxic effects of plastic leachates as a whole remain largely underexplored, particularly for freshwater organisms. We tackled these complexities by monitoring the leaching behavior and ecotoxicity over time of two types of virgin and UV-aged plastic fragment derived from shopping bags conventional low-density polyethylene (LDPE) and compostable polybutylene adipate terephthalate blended with thermoplastic starch (PBAT/TPS). The leaching proceeded for 11 weeks and physico-chemical parameters including pH, dissolved organic carbon, nitrogen and phosphorous species and metals were monitored as well as ecotoxicity testing with freshwater organisms *Lemna minor* and *Daphnia magna*.

Results indicate that leachates from both plastic types inhibited the growth of *Lemna minor*, whereas *Daphnia magna* was unaffected. The growth inhibition in *Lemna minor* may be related to pH changes in the medium induced by plastics. Notably, UV-aged plastics released more organic matter and metals, emphasizing the risks associated with plastic aging.

Overall, these findings highlight the importance of thoroughly examining plastic materials and their leachates to understand their ecological impacts. By expanding knowledge on plastic leachate toxicity in freshwater, this study underscores the urgent need for comprehensive data on the effects of plastic pollution across different ecosystems. Such information is crucial for advancing risk assessments and guiding safer plastic alternatives.

3.23.P-Th337 Seasonal Distribution of Small Microplastics and Microlitter Components < 100 µm in the Inlet of a Drinking Water Treatment Plant

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Microplastics and microlitter, particularly those particles < 100 µm (SMPs and MLCs, respectively), have been observed in seawater and freshwater. According to the One Health Approach and the recent decision of the EU for water for human consumption (Commission Delegated Decision (EU) 2024/1441), it is fundamental to investigate the occurrence and the transport pathways and evaluate the fate of microplastics in raw water to be treated in drinking water treatment plants (DWTPs) and intended for human consumption. This work was supported by ARPAV in the framework of the BSL 6 Project, funded by Veneto Region. An artificial channel that collects and transports the water of the Sile River, meant for human consumption to a DWTP located at Ca Solaro was monitored for a whole year. Raw water was sampled monthly in the inlet of the DWTP using a customized glass water sampler, cleaned and decontaminated before use. The collected water was then stored in decontaminated amber glass bottles and transported to the Institute of Polar Sciences of the National Research Council (CNR-ISP) laboratory, where they were stored at 4°C before the pretreatment and the analysis. A rigorous QA/QC was applied to minimize plastic contamination at any step. All the operations were performed in a clean room ISO 7, which is wholly made of stainless steel and glass, and the air filters do not present plastic polymers (plastic-free clean room). An oleoextraction, previously developed, was employed as pretreatment, and the oleoextracts were filtered on aluminum oxide filters (0.2 µm, 47 mm); a purification step was performed during the filtration to remove all the interferents for the analysis via vibrational spectroscopy. An iN10 Nicolet Micro-FTIR was employed for the identification of the particles and their simultaneous quantification (Particles Analysis). Differences in the abundance and distribution of plastic polymers, plastic additives, and other microlitter components were observed. Plastic additives can be related to the presence of plastic particles and be employed as proxies of plastic polymers. The size distribution showed that the particles were concentrated in the range 20-50 µm in length for most of the polymers and microlitter components observed, although there were some exceptions, such as the particles of polyamide 6 (or nylon, PA 6). These data are crucial for the development of corrective actions and for planning mitigation strategies for plastic pollution.

Disclaimer/Disclosure: This work was supported by ARPAV in the framework of the BSL 6 Project, funded by Veneto Region.

3.24.A Analysis and Monitoring of Per- and Polyfluoroalkyl Substances (PFAS): Challenges, Standardization and Innovative Analytical Approaches

3.24.A.T-01 Navigating the Complex Landscape of PFAS CRMs

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Per- and polyfluoroalkyl substances (PFAS) have emerged as a significant environmental concern due to their persistence and potential toxicity. Accurate and reliable analysis of PFAS necessitates the use of certified reference materials (CRMs). However, the diversity and complexity of PFAS compounds present unique challenges in developing suitable CRMs.

PFAS manufacturing processes often result in products containing a mixture of branched and linear isomers, as well as other organic impurities, water, solvent, and inorganic residues. This complex mixture can significantly impact the accuracy of PFAS quantification in environmental matrices. Contaminants can affect chromatographic and net purity, influencing analytical detector response and compromising the reliability of quantitative analysis. Additionally, different PFAS homologues and isomers may exhibit distinct environmental and toxicological properties. Therefore, reference material producers must provide a range of fully characterized reference standards and labelled internal standards.

This study focuses on the development of PFAS CRMs, including the synthesis, purification, and characterization of isomerically pure, primarily n-isomer PFAS compounds. This presentation will delve into the current state of PFAS CRM availability, limitations, and emerging approaches to address these challenges. By understanding the current landscape and future trends in PFAS CRMs, we can work

towards improving the accuracy and reliability of PFAS analysis.

Forty high-purity PFAS CRMs have been developed and validated to ensure accurate and reliable analysis. These CRMs will significantly contribute to the accurate monitoring of PFAS in environmental and biological samples.

Disclaimer/Disclosure:

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3.24.A.T-02 Quantification of Ultrashort- to Long-Chain Perfluorocarboxylic Acids in Water Samples via Static Headspace Gas Chromatography Coupled to Mass Spectrometry

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Perfluorocarboxylic acids (PFCAs) are one of the most prominent and studied subgroups of per- and polyfluoroalkyl substances (PFASs), which have attracted great interest in environmental and toxicology research due to their intensive use in combination with their persistence, mobility and potential threat to ecosystems and human health.

The standard method to quantify PFCAs in water samples is reverse phase liquid chromatography or, for additional quantification of ultrashort-chain PFCAs (fluorinated carbon chain length of one or two carbon atoms) too, hydrophilic interaction liquid chromatography, in each case coupled with tandem mass spectrometry. However, there are a lot of laboratories using mass spectrometry coupled to headspace gas chromatography systems (HS-GC-MS), usually to quantify a wide range of volatile organic compounds. While PFCAs themselves are difficult to measure with HS-GC-MS in water samples, this challenge can be solved by derivatizing the acids to more volatile compounds, like esters. This approach could enable laboratories using HS-GC-MS to analyse and monitor PFCAs in environmental water samples.

By following this idea, we successfully developed a HS-GC-MS method to quantify PFCAs in ultrapure water. Trifluoroacetic acid (TFA), perfluoropropionic acid (PFPrA), perfluorobutanoic acid (PFBA) and perfluorooctanoic acid (PFOA) served as exemplary analytes of PFCAs, which were derivatized with methanol and concentrated sulfuric acid to form methyl esters. The method was optimized by varying different preparation and measurement parameters, leading to limits of detection and quantification in the ppt-range for all four analytes. The method was then tested on various real water samples, including tap and bottled water, groundwater, landfill leachate, treated wastewater and impinger water from a sewage sludge incineration experiment. Furthermore, snow samples from different locations were analyzed too. TFA was the most frequently detected PFCA, with concentrations in the ppt and low ppb range. The detection and quantification of the remaining three PFCAs vary with the sample types and sampling location.

Our results show that HS-GC-MS is suitable, and enables laboratories with this technique to quantify PFCAs in water samples.

3.24.A.T-03 Advancing PFAS Identification: Pyrolysis-GC-HRMS Analysis of Side-Chain Fluorinated Polymers and Fluoropolymers in Consumer Products

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Per- and polyfluoroalkyl substances (PFAS) have long been utilized in a wide range of consumer and industrial applications due to their unique properties. Non-polymeric PFASs with low molecular weights have been the predominant focus of research and regulatory bodies. However, polymeric PFAS comprise the bulk of PFAS products but have been little studied. Among these, side-chain fluorinated polymers (SCFPs) and fluoropolymers are extensively used due to their unique hydrophobic and oleophobic properties, yet their identification remains elusive due to their polymeric nature and lack of standardized analytical techniques.

Pyrolysis-GC coupled with high-resolution mass spectrometry (HRMS) is a novel technique that can identify bulk polymer types with minimal sample preparation. The pyrolysis step breaks down the fluoropolymer/SCFPs into its constituent monomers. The pyrolysis degradation products indirectly provide a structural elucidation of unknown polymeric PFAS compounds in the sample.

This study demonstrates the use of Py-GC-HRMS for the identification and differentiation of polymeric PFAS. Two fluorotelomer acrylate-based SCFPs (C6-SCFP and C8-SCFP) and seven fluoropolymers were evaluated as reference materials. Characteristic pyrolysis products were identified for each material, with indicator ions enabling reliable detection in consumer products. Pyrolysis at 700 °C revealed

distinctive retention times and mass spectra, allowing for the development of a comprehensive database of polymer-specific markers. These marker compounds were validated in environmental and consumer product matrices, enabling the differentiation of fluoropolymers and SCFPs. A database of retention times, accurate masses, and fragment ion patterns was established, enhancing the reliability of Py-GC-HRMS for PFAS detection.

The methodology was applied to 22 textile samples and 10 cosmetics samples, representing diverse applications of polymeric PFAS. In the textile samples, PFAS were identified in 17 cases, with SCFPs predominantly detected in high-fluorine-content materials like GORE-TEX coatings. The cosmetics analysis revealed the presence of polymeric PFAS in several formulations, indicating their widespread use in consumer products. The ability to identify and quantify these polymers underscores the sensitivity and specificity of Py-GC-HRMS, addressing a critical gap in PFAS analysis.

3.24.A.T-04 Fluorine Elemental Analysis in Complex Sample Matrices: Prospects and Challenges for PFAS Analytics

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The European Union aims to impose restrictions on the use of per- and polyfluoroalkyl substances (PFAS) across various products and processes. This ambitious proposal was put forward by Denmark, Germany, the Netherlands, Norway, and Sweden in January 2023. It is noteworthy for its unprecedented scope, encompassing over 10,000 substances. The attempt to regulate such a wide-ranging class of substances, which possess vastly different properties, presents new challenges for analytics. To effectively monitor PFAS in consumer and industrial products at trace-level concentrations, there is a need to develop suitable analytical techniques. Current methods predominantly target environmental samples, particularly aqueous matrices, and are limited to a narrow range of analytes. Sum parameters and fluorine elemental analysis are promising attempts to address some of the challenges in PFAS analytics.

This contribution aims to demonstrate the role of fluorine elemental analysis within an analytical workflow for PFAS assessment. However, performing elemental fluorine analysis of PFAS compounds presents challenges within existing workflows, particularly with respect to sample preparation and fluorine detection using atomic spectrometry. Herein, the authors will discuss the prospects and challenges of total fluorine determination as a screening tool for PFAS in complex sample matrices.

Therefore, fluorine-specific graphite furnace atomic absorption spectrometry (GF-AAS) is evaluated as a method for trace-level fluorine analytics and compared to existing technologies. To extend the diversity of sample matrices that can be analyzed, a broad range of digestion techniques, including microwave-assisted digestion, pyrohydrolytic combustion, and various peroxide fusion setups, were evaluated. By combining these sample preparation approaches with GF-AAS, a novel analytical strategy for fluorine elemental analysis is proposed.

3.24.A.T-05 PFAS Sequencing: A Series of Simple Chemical Transformations for Structure Elucidation of Unknown PFAS

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Substances with perfluoroalkyl structural elements undergo oxidation in persulfate solution, so that perfluorinated part remains largely intact and non-perfluorinated parts get removed. The stable end-products are perfluorinated carboxylic acids (PFCA) with the same or 1-3 CF₂-groups shorter chain length than in the substrate. A popular TOP assay is based on such oxidation.

We hypothesized that derivatization of a PFCA, followed by oxidation can also lead to shortening of a perfluorinated chain. The approach can serve three purposes: (i) synthesis of yet unknown shorter PFCAs from known longer ones, (ii) structure elucidation of unknown longer PFAS based on the structure of known shorter ones, (iii) conversion to derivatives with a molecular ion for sensitive measurement or structure elucidation.

There was a need in a particular PFAS, the perfluoromethoxyacetic acid (12-PFECA); we have designed and executed a series of transformations towards this target.

All reactions employed in this proof-of-concept study can be carried out on micro- or nanoscale with the standard equipment of a trace analysis lab.

Briefly, we converted acids to their methyl esters and further to the 2-aminoethylamides. The amides upon oxidation yielded shorter PFECAs, indeed. The required 12-PFECA was obtained from two precursors, 14- and 122-PFECAs, which allowed to establish its structure unequivocally. A variety of additional GC- and LC-amenable derivatives was prepared. These derivatives, unlike certain parent PFECAs, produce intense molecular ions. The newly synthesized PFECAs can be further transformed to even shorter ones, thus PFAS sequencing was successfully demonstrated.

3.24.B Analysis and Monitoring of Per- and Polyfluoroalkyl Substances (PFAS): Challenges, Standardization and Innovative Analytical Approaches

3.24.B.T-01 Environmental Fingerprints of Fluorotelomer-Based Substances by GC-Based Target, Suspect, and Non-Target Analyses Surrounding Fluorochemical-Related Industries in Korea

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As a family of high-performance chemicals, per- and polyfluoroalkyl substances (PFASs) have been extensively used in textiles, packaging materials, and grease repellants. Neutral PFASs (n-PFASs), including fluorotelomer alcohols (FTOHs), perfluorooctane sulfonamides (FOSAs), and perfluorooctane sulfonamidoethanols (FOSEs), serve as key intermediates in the production of fluorinated polymers. These compounds, referred to as precursors, have the potential to transform into various PFASs through atmospheric oxidation and biotransformation processes. Despite increasing concerns to precursors, the environmental behavior of n-PFASs encompassing their emission, transport, and degradation remains poorly understood compared to the well-known regulated PFASs. Air, sediment, and industrial wastewater samples were collected from five fluorochemical-related industrial facilities of Korea (textile, paper mill, and semi-conductor manufacturing) in 2020. Thirteen neutral PFASs were determined in air and sediment samples using gas chromatography and tandem mass spectrometry (GC/MS/MS). Suspect and non-target analysis with a GC and time of flight mass spectrometry (GC/TOF-MS) was attempted to identify other fluorinated compounds. Suspect and non-target analyses identified and semi-quantified 11 novel and emerging fluorotelomer-based substances, including fluorotelomer iodides (FTIs) and fluorotelomer methacrylate (FTMAC). Elevated concentrations of n-PFASs were observed in air and sediment samples near industrial facilities, indicating that n-PFASs are primarily emitted into the atmosphere and subsequently subject to deposition or degradation during atmospheric transport. Matrix-dependent profiles were observed in the form of predominance of shorter- and longer-chained n-PFASs in air and sediment samples, respectively. These findings suggest that air and sediment act as mobile and secondary sources of various PFASs in the environment. Additionally, fluorinated polymer-producing and consuming facilities showed distinct emission patterns of n-PFASs, underscoring the need for tailored strategies to monitor and mitigate PFAS emissions across different industries. Our studies highlight the importance of a comprehensive approach with GC-based target, suspect, and non-target analyses to better understand the environmental fate of PFAS from potential sources and to characterize contamination profiles of PFAS in multiple environmental matrices.

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3.24.B.T-02 Screening and Migration Potential of Per- and Polyfluoroalkyl Substances in End-of-Waste Plastics and Textiles

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The End-of-Waste (EoW) procedure, as defined in the European Waste Framework Directive (2008/98/EC), aims to provide a legal framework for remarketing waste-derived materials. However, the establishment of EoW criteria for certain recycled goods remains under debate, largely due to the lack of scientific consensus on the risks these materials may pose to human health and ecosystems. A key concern is the potential accumulation of contaminants, such as per- and polyfluoroalkyl substances (PFASs), in recycled raw materials compared to virgin counterparts. This study focuses on recycled plastics and textiles classified as EoW in Italy, which currently lack PFASs monitoring requirements despite their widespread reintegration into the circular economy.

To address this gap, a testing framework was developed to investigate the occurrence and migration potential of PFASs in recycled plastics and textiles. More than 30 samples of plastic polymers (e.g., polypropylene, polyamide, polyoxymethylene, polycarbonate, and acrylonitrile-butadiene-styrene) and 10 samples of textiles (e.g., polyethylene and natural fiber mixtures) were sourced from post-consumer and post-industrial waste streams in Italy. These materials were cryomilled to fine powders and analyzed for over 40 PFAS compounds. Migration tests simulated potential human exposure routes, including food

intake (EU Regulation 10/2011), oral exposure (EU JRC guideline EUR 19899), and dermal exposure using artificial sweat and sebum. Experimental conditions were tailored to maximize release potential while avoiding cross-contamination.

Preliminary findings, to be discussed, aim to provide a comprehensive screening of PFAS levels in recycled materials and assess their migration potential under intended use scenarios. This research is expected to offer valuable data to inform future restrictions and refine EoW criteria, supporting a safer integration of recycled materials into the circular economy.

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3.24.B.T-03 Semi-Quantitative Nontarget Screening – The Next Step to Characterize an AFFF Contaminated Site

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The analysis of PFAS in environmental samples is very challenging because of their diverse physical/chemical properties and the high number of individual PFAS compounds. Target analysis is not feasible for a comprehensive characterization as only a few PFAS can be quantified and hence the total PFAS burden is often drastically underestimated. Therefore, nontarget screening (NTS) approaches are highly attractive and provide promising results. On the other side NTS is per se not quantitative. On an AFFF contaminated soil site due to a large fire event in 2008 we identified 124 PFAS and their transformation products from 42 subclasses at confidence levels for identification between 1 and 3. These included anionic, cationic, and zwitterionic substances with 3 to 14 perfluorinated carbon atoms. Since for a large part of the identified PFAS authentic analytical standards are missing, only 28 PFAS could be quantified based on standard calibration functions. The signal intensity of the analytes alone is completely unsuitable for quantitative estimation, as this depends significantly on the structure, functional groups, chromatography (solvent composition) and mass spectrometry conditions in the electrospray process and varies over several orders of magnitude for different analytes at comparable concentrations. We therefore developed and applied a semi-quantification method based on matrix-matched average calibration curves of suitable authentic standards taking four different functional and/or ionization groups into consideration: carboxylic acids, sulfonic acids, sulfonamides, and cationic PFAS. Matrix effects in the soil extracts on the ionization efficiency have been considered by a standard addition approach. The results reveal that semi-quantification of PFAS concentrations for specific compound classes can be achieved at a median prediction error of 2.2X, at a range between 1.3X and 5X. This showed that in the topsoil of the contamination cationic and zwitterionic PFAS dominate and that semi-quantified PFAS more than doubled the sum concentration of the quantified PFAS in soil (7.3 µg/g) and the total sum finally explains completely the extractable organic fluorine (EOF) of the contamination. In conclusion, these findings have considerable consequences for the assessment of mitigation measures and remediation approaches.

3.24.B.T-04 Assessing the Added Value of the TOP Assay and AOF in Water Management

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Per- and polyfluoroalkyl substances (PFAS) comprise several thousands of compounds; however, only a relatively small number of these compounds are routinely monitored. Alternative analytical methods, such as the total oxidizable precursors (TOP) assay and adsorbable organic fluorine (AOF), have been recently utilized to detect a wider range of PFAS; however, both methods have benefits and limitations.

In this study, the TOP assay and AOF were compared to conventional targeted analysis to evaluate their potential added value in water management. The comparison was based on the analysis of surface water and wastewater samples, and a mass balance analysis to determine the amount fluorine (potentially PFAS) detected by each method.

After TOP assay, 13 out of 33 samples showed an increase in ΣPFCA > 50%, and 7 out of 33 and increase > 100%. For samples with relatively low contamination levels, the difference between ΣPFCA before and

after oxidation resulted in negative values, likely due to analytical variability. Targeted precursors did not explain the increase in ?PFCA observed after oxidation, suggesting contributions from precursors excluded from the targeted analysis.

AOF detected the highest amount of fluorine in 15 out of 23 wastewater samples, with concentrations exceeding those measured by targeted analysis by factors ranging from approximately 2 to 100. This suggested that PFAS were potentially missed by targeted analyses, even when including the contribution of precursors detected by the TOP assay. In the remaining 8 samples, targeted analysis (including ultrashort-chain PFAS) measured the highest fluorine concentrations, likely due to poor retention of ultrashort chain PFAS by the sorbent used for the extraction of organic fluorine by the AOF method. In surface water samples, AOF concentrations were up to one order of magnitude higher than those estimated using targeted analysis in all samples except for one.

Overall, results indicated that a substantial portion of PFAS was missed by conventional targeted analysis. AOF detected the largest amounts of organic fluorine, but the gap between AOF and targeted analysis was never adequately explained by the presence of precursors. This suggested that the TOP assay maybe missing a considerable portion of organic fluorine (potentially be PFAS). Future research should focus on the identification of the organic fluorine detected by AOF and the precursors detected by the TOP assay.

3.24.P-Mo266 Monitoring PFASs and Alternatives in Seafood: Analytical Method Development and Effects of Cooking Processes

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Per- and polyfluoroalkyl substances (PFASs) have been widely utilized in consumer goods such as food packaging and nonstick cookware coatings due to their unique physicochemical properties. However, their persistence in the environment and accumulation in food products, combined with known human toxicity, have led to increasing global restrictions on their use. In response, alternative compounds such as Gen-X (HFPO-DA) and F-53B (9Cl-PF3ONS) are being adopted. While these alternatives exhibit reduced bioaccumulation potential, emerging evidence suggests they may pose toxicological risks, highlighting the need for further monitoring and assessment.

In this study, we developed an analytical method using UPLC-MS/MS to simultaneously quantify 20 PFASs, 12 alternative compounds, and 3 metabolites in seafood. Three sample preparation techniques QuEChERS, SPE, and LLE were compared to optimize recovery efficiency and sensitivity. QuEChERS was selected for its superior absolute recovery rates (ranging from 5 133%) across most analyte groups. The developed method showed high selectivity, accuracy (72.5 114.4%), and precision (0.4 16.3%), with detection limits ranging from 0.003 to 0.157 ng/g.

The method was applied to seafood samples to assess PFASs and alternative compounds pre- and post-cooking. Raw seafood showed significant contamination with PFASs, with PFOA, br-PFOS, and L-PFOS as the most prevalent compounds. Cooking processes, such as boiling and baking, reduced PFAS concentrations to varying degrees depending on temperature, duration, and sample preparation, though not consistently across all compounds. For example, PFOS and PFBA concentrations increased 1.6 and 5 times, respectively, during baking, while boiling showed limited reduction, aligning with previous findings.

These results highlight the complexity of PFASs' behavior during cooking and underscore the need for further research to clarify the mechanisms driving concentration changes. This study provides foundational insights into PFASs and their alternatives in seafood, offering critical data to inform biomonitoring and regulatory strategies.

3.24.P-Mo284 enviPath-PFAS: A Publicly Available, Expert-Curated Database and Pathway Prediction System for Biotransformations of Precursors to Persistent Perfluorinated Alkyl Substances (PFASs)

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Per- and polyfluoroalkyl substances (PFASs) are man-made industrial chemicals used in manufacturing processes and commercial products to provide resistance to heat, oil, grease, and water. Unique chemical properties of these substances make them highly persistent in the environment, especially for

perfluorinated compounds. As complex mixtures of PFASs are released into the environment, polyfluorinated precursors can transform into thousands of possible molecular structures and eventually into perfluorinated end products depending on the environmental conditions. It is important for environmental chemists to understand these complex transformation processes to identify intermediate and terminal chemical structures such that comprehensive assessments on PFAS contamination at different sites can be performed.

In this project, we address this need by collecting and disseminating PFAS transformation pathways reported in scientific literature through the publicly available biotransformation database and pathway prediction system, *enviPath* (envipath.org). We developed a PFAS-specific data package in *enviPath* that contains extensive information on biologically catalyzed PFAS transformation pathways in diverse environmental microbial communities such as in soil, sediment, surface water, and activated sludge. Each pathway in the database is annotated with experimental metadata and kinetic information (if available) that can be used to interpret the relationship between environmental conditions and observed PFAS biotransformations.

We additionally used data from our PFAS-specific package to retrain the rule-based pathway prediction models in *enviPath* that were previously developed using mostly non-fluorinated compounds. We show here the performance of our PFAS biotransformation pathway prediction algorithms with the likelihood that each of our studied polyfluorinated precursors will be transformed into a perfluorinated end product in a specific environment. Our database and pathway prediction system in *enviPath* provides scientists with one organized and easily accessible online location to conveniently characterize, predict, and understand environmental PFAS contamination. This information will be essential for future efforts to remediate PFAS contaminated sites as well as for determining the source of PFAS emissions into the environment.

3.24.P-Mo286 Detection, Quantification, and Treatment of Per- and Polyfluoroalkyl Substances (PFAS) in Groundwater (DFEAT-PFAS)

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Per- and polyfluoroalkyl substances (PFAS) are a large group of chemicals used in the formulations of thousands of consumer goods. Because of the recent regulations and restrictions on the use of long chain (C8) PFAS a significant shift in the industry towards short (C4-C7) and ultrashort (C1-C3) chain alternatives has been recognized in the last years. Due to the high polarity and water solubility of ultrashort PFAS, the potential for bioaccumulation is low. However, the high persistence of ultrashort-chain PFAS will result in environmental accumulation, especially in aquatic environments, leading to potential risks for aquatic organisms and increased human external exposure through drinking water. Ultrashort PFAS like trifluoroacetic acid (TFA) are low to moderately toxic to a range of organisms. In the project we are focusing on detecting and removing PFAS, especially ultrashort-chain PFAS from contaminated groundwater. Therefore, we suggest developing and optimizing short- and ultrashort-chain PFAS detection, quantification, and removal. We will design passive sampling devices, which can collect and monitor the temporal profile of PFAS species in groundwater. This will allow us to analyze PFAS contaminations in German and Israeli groundwater using state-of-the-art novel analytical techniques. In addition, contaminated groundwater will be treated via a two-stage process, designed to concentrate the relatively low PFAS concentrations by novel membrane processes including closed-circuit reverse osmosis (CCRO) and mixed matrix composite nanofiltration membranes (MMCM). Afterwards the rejected streams, containing higher concentrations of PFAS will be treated by coagulation and the remaining PFAS adsorbed onto carbonaceous nanomaterials (CNMs).

3.24.P Analysis and Monitoring of Per- and Polyfluoroalkyl Substances (PFAS): Challenges, Standardization and Innovative Analytical Approaches

3.24.P-Mo266 Monitoring PFASs and Alternatives in Seafood: Analytical Method Development and Effects of Cooking Processes

Yeun Seo¹, Junghoan Kim¹, **Younglim Kho¹**, EUNHEE LEE² and Hyeri Jeon¹, (1)Eulji University, "Korea, Republic of", (2)Far-East University, "Korea, Republic of"

Per- and polyfluoroalkyl substances (PFASs) have been widely utilized in consumer goods such as food packaging and nonstick cookware coatings due to their unique physicochemical properties. However, their persistence in the environment and accumulation in food products, combined with known human toxicity, have led to increasing global restrictions on their use. In response, alternative compounds such as Gen-X (HFPO-DA) and F-53B (9CI-PF3ONS) are being adopted. While these alternatives exhibit reduced

bioaccumulation potential, emerging evidence suggests they may pose toxicological risks, highlighting the need for further monitoring and assessment.

In this study, we developed an analytical method using UPLC-MS/MS to simultaneously quantify 20 PFASs, 12 alternative compounds, and 3 metabolites in seafood. Three sample preparation techniques QuEChERS, SPE, and LLE were compared to optimize recovery efficiency and sensitivity. QuEChERS was selected for its superior absolute recovery rates (ranging from 51 to 133%) across most analyte groups. The developed method showed high selectivity, accuracy (72.5 to 114.4%), and precision (0.4 to 16.3%), with detection limits ranging from 0.003 to 0.157 ng/g.

The method was applied to seafood samples to assess PFASs and alternative compounds pre- and post-cooking. Raw seafood showed significant contamination with PFASs, with PFOA, br-PFOS, and L-PFOS as the most prevalent compounds. Cooking processes, such as boiling and baking, reduced PFAS concentrations to varying degrees depending on temperature, duration, and sample preparation, though not consistently across all compounds. For example, PFOS and PFBA concentrations increased 1.6 and 5 times, respectively, during baking, while boiling showed limited reduction, aligning with previous findings.

These results highlight the complexity of PFASs' behavior during cooking and underscore the need for further research to clarify the mechanisms driving concentration changes. This study provides foundational insights into PFASs and their alternatives in seafood, offering critical data to inform biomonitoring and regulatory strategies.

3.24.P-Mo267 Identification of Neutral Fluorotelomer Substances in Sediment Using Gas

Chromatography-Atmospheric Pressure Chemical Ionization-Ion Mobility-Mass Spectrometry

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Despite their large number and broad structural diversity, most research on per- and polyfluoroalkyl substances (PFAS) has focused on a limited suite of polar substances. PFAS that are neutral and (semi-)volatile, in comparison, have received relatively little attention, possibly due to a lack of suitable analytical methods. In the present work, we applied a newly developed method based on gas chromatography-atmospheric pressure chemical ionization-ion mobility-high resolution mass spectrometry for identification of neutral/semi-volatile PFAS in sediments from the Baltic, a Norwegian lake, and the high Arctic. Using a collision cross section (CCS)- and mass-based filtration (i.e., <100 Å² + 20% mass) which takes advantage of the small molecular size of fluorinated substances, we prioritized 34 unknown features in Norwegian lake sediments for further inspection. After scrutiny of in-source fragments and acquisition of reference standards, a series of novel neutral fluorotelomer thiols, disulfides, and alkyl sulfones in sediment were identified at Confidence Level 1-2 for the first time along with their CCS values. The sum semi-quantified concentration of neutral fluorotelomer substances in the Norwegian lake sediment was 96.1 ng g⁻¹ dwt (three times higher than the total concentration of fluorotelomer sulfonates reported previously), with 88.3% attributed to fluorotelomer disulfides. The occurrence of these compounds was further validated in a NIST standard reference marine sediment, as well as in surface sediment from the Baltic Sea and the high Arctic. Only 12:2 fluorotelomer thiol and 8:2/8:2 fluorotelomer disulfide were detected in the Baltic Sea sediment, while fluorotelomer methyl sulfones appeared in almost all samples. Notably, 8:2 fluorotelomer methyl sulfone was detected in one of the Arctic sediment samples, underscoring the widespread environmental distribution and persistence of these substances. This study not only introduces a promising technique for screening neutral and (semi-)volatile PFAS, but reports the identities and CCS values of a group of widespread neutral fluorotelomer substances for further investigation of their sources and health effects.

3.24.P-Mo268 Exploring the Detection Capability of PFAS Using High-Resolution Mass Spectrometry

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Per- and polyfluoroalkyl substances (PFAS) consist of a large group of compounds covering a vast variety

of chemical structures from ionic to neutral PFAS. These compounds have been classified as contaminants of emerging concern due to their widespread distribution and hazardous effects. PFAS are usually analyzed by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) using electrospray ionization (ESI) for both targeted and non-targeted screening (NTS) approaches. However, there is still a need to upgrade of analytical strategies to achieve a wide-scope determination of PFAS (including both ionic and neutrals) and enhance the detection capability of LC-HRMS methodologies. Thus, this work aims to explore mobile phase additives and acquisition methodologies to extend and enhance the PFAS coverage combining atmospheric pressure ionization sources such as ESI, atmospheric pressure chemical ionization (APCI) and photoionization (APPI).

First of all, the influence of the organic modifier (acetonitrile or methanol) as well as different mobile phase additives to enhance specific ionization mechanisms (i.e., electron capture, adduct formation, deprotonation) were evaluated for each ionization source. For instance, the ionization of PFAS by ESI was evaluated using not only conventional additives such as ammonium acetate or ammonia, but also novel additives which have shown to significantly amplify analyte signal, such as ammonium fluoride. Under the most suitable mobile phase conditions for each ionization technique, different acquisition modes involving both full scan and tandem mass spectrometry experiments consisting of data-dependent (ddMS2), variable data-independent (vDIA) or all ion fragmentation (AIF) acquisition were compared. To do so, figures of merit were determined for the validation of the multi-target workflow according to the C/2024/5414 guidelines of the European Commission whereas other parameters such as limits of identification, type I and type II errors were established for the validation of the non-targeted workflow.

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3.24.P-Mo269 A Novel PFAS Workflow for Comprehensive Analysis and Fingerprinting of Contaminated Areas

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PFAS are a widespread environmental problem, with increasing sources of contamination and more regions being affected. This leads to a growing number of samples that need to be analyzed, but this is limited to a subset of known PFAS compounds using a targeted approach. We propose a new PFAS analysis workflow that enables a smart selection of samples for increasingly detailed analysis, demonstrated with an example of soil samples.

When mapping PFAS contamination at a site, soil or other matrices must be collected at multiple locations, depths, and potentially at different time points. This results in an expanding sample set, but not all are necessary for obtaining a comprehensive view of contamination. Soil (or other) samples are screened using ambient ionization (DART) by picking up the soil with a glass capillary and analyzing it directly by holding it in the DART source. Mass spectra are then processed with data treatment methods such as mass defect filtering and peak identification using an in-house database. This initial screening enables rapid classification of soil samples into high, medium, or low PFAS content groups and provides insight into the types of PFAS present, distinguishing between different contamination patterns.

Based on this screening, selected samples are extracted and analyzed with a targeted LC-MS/MS method to quantify up to 92 specific PFAS compounds. The DART results hereby guide the required sample dilution for accurate analysis. To obtain a broader understanding of PFAS contamination, two additional approaches are applied. First, samples are analyzed for total organic fluorine (EOF or AOF) to assess the overall PFAS content. Secondly, the TOP assay is used to indicate the presence of precursor compounds that can degrade into target PFAS.

By comparing the DART results with targeted quantification, EOF, and TOP assay data, the next steps in the analysis are determined. If necessary, samples can be selected for in-depth non-target analysis, which provides more detailed information about PFAS precursors, degradation products, and other PFAS compounds not included in the targeted list.

This workflow provides a detailed and flexible approach to PFAS analysis, allowing for an efficient yet comprehensive evaluation of contamination at a site. By making smart preliminary sample selections, it

enables the identification of key contamination patterns, correlations, and predictions, ultimately improving our understanding of PFAS dynamics.

3.24.P-Mo270 Enhancing Per- and Polyfluoroalkyl Substance (PFAS) Analysis Efficiency in Solid and Liquid Matrices Using Automated Online SPE and LC-MS/MS

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The environmental impact of per- and polyfluoroalkyl substances (PFAS) remains a pressing issue due to their persistence, bioaccumulation, and associated health risks. Efficient and accurate analysis of PFAS is crucial for regulatory development work and environmental protection. Given the widespread concern and focus on PFAS, precise measurement in environmental, biological, and food samples is essential for accurate exposure assessment, risk evaluation, and understanding their fate.

The gold standard for many PFAS analytes is liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS). Sample preparation is essential to achieve ever-decreasing target limits of quantification (LOQs), but these techniques can be quite tedious and time-consuming. Automation of analytical workflows is key to enhancing efficiency and sustainable practices, eliminating human error, and reducing potential contamination during sample handling.

This study demonstrates a fully automated analytical approach for solid and liquid environmental matrices. The workflow comprises solid-liquid extraction, dispersive carbon clean-up and online SPE. A modular robotic sample handling system, is directly linked to the online SPE LC-MS/MS analytical system. The entire workflow is specifically designed and tailored for the analysis of PFAS, ensuring no background contribution from the analytical system. Liquid samples or extracts are automatically injected into a miniaturized online SPE system with an integrated cartridge handling system, directly coupled to the LC-MS/MS system. The methodology encompasses over 40 analytes, following analytical procedures described in US EPA method 1633 and complies with European/ German standard methods DIN 38407-42 DIN and EN 17892:2024.

The implementation of this fully automated analytical method for PFAS analysis in environmental samples significantly enhances laboratory efficiency and accuracy. By reducing human error and contamination risks, this approach ensures more reliable data for regulatory compliance and environmental protection. Our findings underscore the importance of automation in advancing analytical chemistry, promoting greener and more efficient practices in routine laboratories.

3.24.P-Mo271 Quantification and Identification of PFAS substances in Dilute Solutions by 19F-NMR Spectroscopy

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We herein present and outline a set of methodology to identify and quantify (poly)-fluorinated organic compounds down to very low limits of detection by 19F-NMR spectroscopy. 19F-NMR spectroscopy has recently emerged as a versatile tool for screening and identification of fluorinated compounds for pharmaceutical R&D and for environmental analytics.

Since the spectroscopic properties of 19F are very similar to that of 1H in many regards, 19F-NMR can be applied at most modern high-field NMR-systems in a routine without the need for a special technical setup. However, to achieve very high sensitivity in order to detect sub-ppm concentrations in dilute solutions, cryo probes are necessary. We report our method development for a 700 MHz Avance IIIHD spectrometer equipped with a 5 mm TCI-cryoprobe, showcasing limits of detection for C-F3 containing compounds of about 25 ppb for certain applications. Excellent spectral resolution allows the identification of a series of small organic molecules as a fingerprint of their 19F chemical shift, based on database evaluation. Further, the spectral resolution also allows for a targeted quantification of different F containing compounds even in mixtures and formulations.

We highlight the applicability of this approach in close collaboration with other analytical methods and outline a strategy to gain analytical insights into challenging sample matrices.

3.24.P-Mo272 PFOA Sensor

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PFAS, or per- and polyfluoroalkyl substances, are man-made chemicals with a unique structure featuring a hydrophobic fluorinated carbon chain linked to a hydrophilic functional group. This structural peculiarity

grants PFAS compounds both hydrophobic and oleophobic properties, making them valuable in various industrial and household applications. However, the robust C-F covalent bond in PFAS leads to their persistence in the environment, earning them the moniker "forever chemicals."

Among PFAS compounds, PFOA stands out globally due to its extensive use and remarkable environmental persistence, with an estimated half-life of around 40 years. This longevity allows PFOA to accumulate in organisms, posing significant health risks such as immunotoxicity and carcinogenicity. PFOA also remains a stable byproduct of many precursor perfluorinated compounds, necessitating the development of analytical techniques for its detection in water sources.

The prevalent methods for detecting PFOA primarily involve chromatography techniques like high-performance liquid chromatography (HPLC) and gas chromatography (GC). In 2009, the U.S. EPA introduced a liquid chromatography-mass spectrometry method (Method 537) for PFOA detection in drinking water. While these chromatography methods offer precision, they are often cost-prohibitive and require skilled operators due to their complexity and time-consuming procedures.

To address the drawbacks of chromatography, alternative detection methods have emerged, notably colorimetric and fluorescent techniques. Colorimetric sensors are user-friendly and cost-effective, suitable for rapid on-site testing, while fluorescent sensors offer heightened sensitivity and specificity, ideal for detailed analyses and detecting low concentrations of PFOA. Consequently, this study focuses on developing a swift fluorometric approach for PFOA determination.

This study found an innovative method pertains to fluorescence detection using C-12 nanoclusters to detect PFOA. By mixing aqueous C1 and C2 precursor solutions to create C-12 nanoclusters, a strong fluorescence intensity is achieved. The presence of PFOA can quench this fluorescence, offering an efficient and economical detection method for PFOA.

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3.24.P-Mo273 Ultrashort Chain and Highly Polar PFAS Analysis: Development of Methodology for the Determination of Residues in Human and Environmental Samples *Stephen Brewin, Environmental Sciences, Labcorp, Indianapolis, United Kingdom*

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic organofluorine compounds that have become a concern due to their persistence in the environment following extensive use over the last few decades. Due to their high degree of chemical stability, as well as their widespread use, PFAS now are increasingly being detected in the environment and appear to persist for extended periods of time.

Furthermore, exposure to these chemicals in everyday objects, such as packaging and cookware, as well as within certain areas of industry, has led to the apparent bioaccumulation in certain areas of the population. Because of the concerns over health and environmental issues associated with PFAS, there has been a need to be able to monitor the occurrence of these materials in a range of matrix types.

Ultrashort chain PFAS analytes are small and highly polar compounds, with very short carbon chain lengths. These have become of considerable concern, alongside their longer chain counterparts. As a result, there is an increasing need to be able to quantify these materials in a range of environmental and biological samples, in order to assess the exposure of the population to these chemicals.

There are numerous approaches to analysing a range of these chemicals in a number of different matrix types, and this area of analytical chemistry is constantly evolving as new PFAS chemicals are identified and added to the list of those where methods already exist.

The work described in this poster summarises a project to develop and validate an LC-MS/MS method for the analysis of a typical suite of ultrashort chain PFAS chemicals in environmental samples (surface water) as well as in body fluids (plasma). A simple and robust method was required, that could be easily applied to routine analysis at low concentrations, using commonly available equipment and approaches. There was also a requirement to be able to add additional short chain PFAS analytes to expand the method as the need arises.

The method development work for the analysis of PFAS chemicals in the tested matrices is still ongoing and is based on common laboratory approaches, with quantitation performed by LC-MS/MS. This poster will present the work performed to date.

3.24.P-Mo274 Who Doesn't Love Dilute and Shoot? Robust Methodologies for Analyzing PFAS in Varying Matrices

Tom Hey, PerkinElmer, Canada

Over the past decade, awareness and concern about per- and polyfluoroalkyl substances (PFAS) has grown and led to more in-depth analysis of these compounds and the effects that they can have on human health.

Commonly extensive cleanup and/or concentration steps are employed to meet the detection limits defined in regulated methods. We have found due to the robustness of our QSight LCMSMS source we are able to avoid SPE and utilize a much more simple approach of dilute and shoot and still meet the detection limits needed for methods such as EPA 1633.

3.24.P-Mo275 Evaluation of DESI Mounted on Q-ToF and TQ Mass Spectrometers to Image PFAS Compounds in Different Matrices

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Per- and polyfluoroalkyl substances (PFAS) are a well-known group of chemicals that have a variety of commercial and consumer uses. They have been found to be highly persistent and can accumulate in humans, animals and the environment. Traditional analysis of PFAS has focused on determination with LC-MS/MS, often laborious SPE techniques are required before analysis of solid matrices, such as food, plants or animal tissue to assess exposure routes. Here we propose the use of desorption electrospray ionization (DESI) combined with a Q-ToF operating in full scan and using a high-performance tandem quadrupole mass spectrometer to perform mass spectrometry imaging (MSI) experiments.

A mixture of PFAS compounds (PFAC30PAR, Wellington Laboratories) containing among others PFHxS, PFOS, PFOA, PFNA, PFDA, FBSA, 6.2FTS, PFBS, FOSA, was used to evaluate the levels of detection between different systems such multi-reflecting Q-ToF (MRT), cyclic IMS Q-ToF and tandem quadrupole mass spectrometers. The mixture was diluted in 50:50 MeOH/H₂O. Lentils were grown wet cotton balls containing different concentrations of PFAS mixture and let grown with natural light for 14 days.

Samples were analyzed using a multi-reflecting Q-ToF (MRT), a Cyclic IMS Q-ToF (in TOF and IMS modes) and a TQ in negative ionisation mode.

From the DESI MRM TQ data, 1-3 transitions were set for 26 of the most important PFAS compounds to evaluate the best MRM transition for further MSI experiments. Only PFTrDS compound was not detected at the highest level. 77% of the PFAS compounds that were targeted were detected at 1-10 ng/mL.

In full scan MS, also 77% were detected at 1-10 ng/mL or lower when analysed using the Cyclic IMS vs. 61% on the MRT and identified with a mass accuracy lower than 500 ppb.

Interestingly the PFAS compounds have a much faster arrival time by ion mobility separation compared to singly charged tissue or solvent background peaks, enhancing the overall S/N.

Further experiments using the targeted MRM DESI TQ was performed where radishes were incubated in a control solution and a PFAS mixture solution at 10 ng/mL. The most efficiently ionising PFAS, and coincidentally those regulated in Europe, such as PFOS (498.9>80.2), PFOA (412.9>169), PFNA (462.9>219) and PFHxS (398.9>80.1), were detected from the PFAS incubated radish section. The localisation of PFOS was on the outer ring of the radish whereas the other three PFAS were distributed more evenly throughout the section.

3.24.P-Mo276 Harnessing the Power of Mass Spectrometry and Automation to Reduce Sample Size, Sample Preparation Time and Increase Laboratory Efficiency

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US EPA Method 1633 has become the foundational method for analysis of PFAS in non-potable water matrices, soils, biosolids and tissues in the United States. The method consists of sample preparation using weak anion exchange (WAX) solid phase extraction (SPE) with graphitized carbon black (GCB) clean up. This EPA method is a performance based method allowing for changes to be made as long as data quality equivalency is demonstrated. With this method becoming very much in demand, laboratory throughput and efficiency must be considered for maximum laboratory success. Automation and instrument sensitivity are investigated as avenues to decreasing required sample size and therefore increasing

laboratory efficiency.

A highly sensitive LC-MS/MS system was utilized for this analysis, allowing for reduction in sample volume size while still achieving equivalent results and method detection limits to the method. Sample volumes tested ranged from 500 mL (the recommended volume in EPA 1633) down to 50 mL using an automated solid phase extraction (SPE) system for sample preparation of water samples to eliminate the need for human interaction during the sample preparation process. To make the sample preparation process fully automated, a stacked dual layer cartridge containing both WAX and GCB chemistries was utilized instead of having to perform the GCB step manually.

Equivalency was established by demonstrating that all quality control guidelines were achieved including retention time stability, ion ratio stability, ongoing calibration verification, and recovery. Equivalency of the automated SPE system and stacked dual layer cartridge is also demonstrated as a complete workflow. A method detection limit study will demonstrate equal or lower MDLs using the automated, reduced sample volume approach. Additionally, a wastewater certified reference material was processed in an equivalent reduced sample volume and was within all certified levels for the 40 PFAS covered by EPA 1633.

3.24.P-Mo277 Identification and Quantification of Ultra-Short Chain and Short Chain Per and Polyfluorinated Alkyl Substances (PFAS) Using Ion Ratios With Low Mass Product Ions in a Single LC-MS/MS Run

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As the environmental impact and health hazards of legacy long chain (C8) PFAS emerged, they started to be substituted for short (C4-C7) and ultrashort chain (C3) PFAS in manufacturing. The belief was these would have less of an impact but initial evidence suggests that there are still concerns regarding their effects. The analysis of the carboxylate PFAS with chain lengths C3-C5 can be problematic in terms of positively identifying the analyte due to the lack of a second product ion with suitable intensity to use for ion ratio calculations to meet common identification criteria, especially PFBA. The use of m/z 19 is possible and this response can be significantly improved using a modified high performance tandem quadrupole mass spectrometer.

For a number of short and ultrashort chain PFAS, predominately carboxylates with a carbon chain length of 5 or less (PFPrA, PFBA, PFPeA), there is limited product ions to use for identification purposes. Often the confirmation fragment for PFPrA and PFPeA used is m/z69 but this is not an option for PFBA. Work has been carried out on a high performance negative ion tandem quadrupole mass spectrometer to show that the detection of a fluoride ion (m/z 19) was possible, with the ion ratios measured for PFPrA, PFBA and PFPeA were 0.023, 0.027 and 0.163. Initial work demonstrated that for PFPeA using 263>19 showed a significantly higher response versus the alternative transition 263>69.

3.24.P-Mo278 Ultra-Sensitive PFAS Analysis According to EU Regulations in Drinking Water

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PFAS are highly present in the public and pose a threat to mankind and nature. Manufactured since the 1940s as water and grease repellents in consumer products, they are considered forever chemicals. With their persistent, bio-accumulative, toxic (PBT) properties and ubiquitous presence in the environment and organisms, there is mounting evidence that exposure to PFAS may generate adverse health effects. EU regulations exist for the routine monitoring of certain PFAS in water and food with legal limits in sub-ppb range for the individual compound. Reaching these limits in a routine and robust manner is a challenge even for triple quadrupole systems of highest performance. Presented is a method for the routine targeted analysis by a novel, fast, robust and highly sensitive triple quadrupole which covers and exceeds the current EU regulations for reliable and confident PFAS quantification in water and food.

A standard list of 40 PFAS was used for the performance evaluation. An Elute HT UHPLC was coupled to an EVOQ DART-TQ+ (both Bruker, Bremen, Germany). The total runtime of the method incl. equilibration was 13.2 min with a gradient of 2 mM ammonium acetate in water and methanol on a Bruker Intensity Solo 2.0 100x2.0 mm column and a Restek 50x2.0 mm delay column. The TQ was operated in both polarities with source parameters and MRM transitions optimized. The scan speed was automatically calculated per compound with a minimum number of 12 spectra per peak and a dynamic window of 0.6

min per analyte.

Mobile phase compositions were optimized, different gradients and flow rate tested, and the equilibration time at the beginning of the injection to avoid accumulation. Typical LOQ reached for all PFAS were in the range of 0.1-0.5 ppt. These limits are 10x lower than those required by the EU Drinking Water Directive (EU 2020/2184). Linearities had typical values for $R^2 \geq 0.999$. The system shows a high robustness with much reduced contamination and crosstalk for multiple injections. The method represents a robust, highly sensitive and fast analysis of PFAS according to and exceeding the current EU regulations.

3.24.P-Mo279 Expanding the Analytical Toolbox for Environmental Monitoring: Volatile PFAS Analysis in Surface Water Using Triple Quadrupole GC/MS

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Poly- and perfluoroalkyl substances (PFAS) are persistent, bioaccumulative pollutants linked to adverse environmental and human health effects, including endocrine disruption, immunosuppression, and organ toxicity. While regulatory frameworks have evolved for many PFAS compounds detected using LC/MS, methods for volatile PFAS remain underdeveloped. These chemicals, capable of volatilization and atmospheric transport, represent a unique risk due to their mobility and potential for inhalation exposure. Monitoring volatile PFAS in surface waters is essential for identifying contamination pathways and mitigating environmental risks.

This study addresses the need for sensitive, reliable methods for analyzing volatile PFAS, such as fluorotelomer alcohols (FTOHs), perfluoroalkane sulfonamides (FASAs), fluorotelomer acrylates (FTACs), fluorotelomer acetates (FTATs), perfluoroalkyl iodides (PFIs), and related compounds, using advanced triple quadrupole GC/MS technology. Leveraging an innovative second-generation high-efficiency ion source, the method achieves trace-level detection. The approach also benefits from robust chromatographic separation and precision calibration, providing high sensitivity and excellent linearity across a wide concentration range.

Surface water samples were analyzed to evaluate both contamination levels and the broader applicability of the method. Preliminary results demonstrate high sensitivity with low detection limits enabling tools for understanding the prevalence and distribution of volatile PFAS.

This work emphasizes the value of complementary GC/MS techniques alongside established LC/MS protocols and highlights the potential of GC/MS to enhance environmental monitoring frameworks, bridging gaps in volatile PFAS detection and informing regulatory advancements.

3.24.P-Mo280 Analysis of Ultra-Short PFAS using the Latest LC/TQ

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A poster presentation will highlight the latest analysis of Ultra-Short PFAS using LC/TQ.

The poster will focus on meeting the challenges of analysing Ultra-Short PFAS in real water matrices, including bore hole water, river water and drinking water. The compounds analysed include; TFA (Trifluoroacetic Acid), PFPrA (Perfluoropropanoic Acid), PFBA (Perfluorobutanoic Acid), DFA (Difluoroacetic Acid), PFMeS (perfluoromethanesulphonic acid), PFETs (perfluoroethanesulphonic acid) and PFPrS (perfluoropropanesulphonic acid).

There will be information on how to address the common challenges including how to: reduce background interferences, retain these polar compounds using HILIC columns, reaching the low limits of detection required using the Agilent 6495D LC/TQ, reducing and removing suppression and enhancement of real water samples. The method highlighted will demonstrate how these challenges have been overcome and highlight real sample data, showing recovery and bias performance.

3.24.P-Mo281 Automated Workflow for PFAS Quantitation in Seafood: Enhancing Precision, Efficiency, and Compliance

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Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals extensively used in various industrial and consumer products due to their resistance to heat, water, and oil. These properties contribute to their persistence in the environment, where they can accumulate in aquatic ecosystems and contaminate marine

life, leading to potential human exposure through seafood consumption. The US FDA, EFSA, EURL POPs, and AOAC have established guidelines and methods to monitor and control PFAS levels in seafood to protect public health.

A primary challenge in PFAS analysis is detecting trace levels in complex food matrices like seafood. Traditional methods involving QuEChERS extraction and solid-phase extraction (SPE) cleanup are labor-intensive and prone to errors, requiring skilled analysts to ensure accuracy and reliability. This study aims to address these challenges by developing a fully automated workflow for the quantitative analysis of PFAS in seafood using the Agilent PAL3 RTC-6495D triple quadrupole LC/MS system.

The automated workflow integrates solvent extraction, QuEChERS salting-out, and μ SPE cartridge cleanup, performed by the PAL3 System, with data analysis conducted on the LC/TQ system in parallel mode. This method was evaluated for its performance against various regulatory requirements, focusing on linearity, sensitivity, accuracy, repeatability, and reproducibility.

Results demonstrated excellent linearity ($R^2 \geq 0.99$), high sensitivity with MDLs and LOQs meeting stringent regulatory criteria. The method's repeatability and reproducibility were confirmed with RSDr and RSDR values well within acceptable limits, demonstrating superior accuracy and precision across multiple PFAS analytes. The automated system significantly reduced manual intervention, minimizing human error and enhancing the precision and reliability of the analysis.

In conclusion, the fully automated workflow developed on the Agilent PAL3 RTC-6495D LC/TQ system offers a reliable, efficient, and high-throughput solution for PFAS analysis in seafood. This approach not only meets but exceeds regulatory requirements, providing a robust tool for routine laboratory operations and advancing the field of environmental and food safety analysis.

3.24.P-Mo282 Comparing Data Dependent and Data Independent Workflows for Per- and Polyfluoroalkyl Substances Analysis of NIST Interlaboratory Study Samples

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FluoroMatch Modular and FluoroMatch Visualizer are open-source tools that simplify suspect and nontarget screening of PFAS compounds. FluoroMatch Modular processes data files that have been extracted using vendor software. It uses a systematic scoring framework to communicate confidence for every feature, alongside reporting confidence levels via the Schymanski schema. IonDecon is a recent enhancement to FluoroMatch. It deconvolutes Data Independent Acquisition (DIA) All Ions data files to incorporate only those fragments correlating with precursor ions. This produces a Data Dependent Acquisition (DDA) formatted output file. We received the samples through the NIST PFAS

Interlaboratory Studies and wanted to use the data to evaluate the capabilities for FluoroMatch IonDecon to process DIA file types like All Ions fragmentation. They consisted of 3 methanolic solution with sample A containing a set of PFAS standard, sample B a methanolic dilution of two aqueous film-forming foam (AFFF) commercial solutions and sample C a methanolic extract of an AFFF-impacted soil amended with an analytical standard of a single PFAS in methanol, respectively. The sample was injected four times for iterative exclusion information-data dependent analysis (iterative MS/MS). The instrument was an ultra-high-performance liquid chromatography (UHPLC) system connected to a quadrupole time-of-flight mass spectrometer (Q-TOF MS). Blanks were acquired every other injection for blank filtering. PFAS were detected in negative electrospray ionization mode. Data was acquired from m/z 100-1500, with MS/MS collision energy set to 0 and 40 eV for both AutoMS/MS (DDA) and All Ions (DIA) acquisition.

Once the MS and MS/MS data had been collected, FluoroMatch Modular was used to annotate peaks extracted by MassHunter Explorer. It generates a systematic scoring framework to communicate confidence for every feature. FluoroMatch Visualizer was developed using Power BI Desktop to provide users with customizable graphs, variables, and tables to help with data interpretation. For example, new columns can be added to tables containing information of interest, new plots, and new splicers and filters can be developed. We will compare and contrast the results when processed by these two modes to determine the advantages to each mode relative to sample complexity and matrix interference in identifying the most PFAS without generating significant false positive identifications.

3.24.P-Mo283 Evaluating TD-GC-MS/MS for Measuring PFAS in Air

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Per- and polyfluoroalkyl substances (PFAS) continue to dominate environmental and public health news. As we learn more about their ubiquitous presence in every aspect of life and work analytical chemists continue to adapt monitoring techniques to new matrices and new priority species. Whilst toxicological

data on many volatile PFAS is limited they are able to transform into compounds with known adverse health effects such as perfluorocarboxylic acids (PFCAs) making robust monitoring a priority. Volatile PFAS is a broad term covering many species, from fluorotelomer alcohols (FTOHs) to freons; this can mean extra cost for labs when trying to target different compound classes. Each compound class can have different sources and reasons for requiring monitoring: understanding emissions, consumer product testing, atmospheric monitoring, indoor air quality, vapour intrusion, occupational health monitoring and ensuring PFAS destruction. These multitude of different sample types can also pose a barrier to broadening research or offering more services if a technique cannot easily be applied to other matrices. Beyond the practicalities of sampling, concentration ranges for PFAS in different sample types can vary hugely.

TD-GC-MS/MS offers a flexible approach to sampling different matrices and analysing multiple target species whilst negating the influence of background. With accessories for collecting samples from air, materials, and industrial sources the technique offers a powerful, practical solution to monitoring PFAS from multiple sample types.

In this presentation data will be presented on the performance of the technique overall including detection limits, robustness and repeatability. Analysis of real samples will show how multiple sources can be monitored.

3.24.P-Mo284 envPath-PFAS: A Publicly Available, Expert-Curated Database and Pathway Prediction System for Biotransformations of Precursors to Persistent Perfluorinated Alkyl Substances (PFASs)

Stephanie Rich¹, Christopher Higgins², Damian Helbling³ and Kathrin Fenner⁴, (1)University of Zurich, Switzerland, (2)Colorado School of Mines, Golden, United States, (3)Cornell University, United States, (4)University of Zurich, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland Per- and polyfluoroalkyl substances (PFASs) are man-made industrial chemicals used in manufacturing processes and commercial products to provide resistance to heat, oil, grease, and water. Unique chemical properties of these substances make them highly persistent in the environment, especially for perfluorinated compounds. As complex mixtures of PFASs are released into the environment, polyfluorinated precursors can transform into thousands of possible molecular structures and eventually into perfluorinated end products depending on the environmental conditions. It is important for environmental chemists to understand these complex transformation processes to identify intermediate and terminal chemical structures such that comprehensive assessments on PFAS contamination at different sites can be performed.

In this project, we address this need by collecting and disseminating PFAS transformation pathways reported in scientific literature through the publicly available biotransformation database and pathway prediction system, envPath (envipath.org). We developed a PFAS-specific data package in envPath that contains extensive information on biologically catalyzed PFAS transformation pathways in diverse environmental microbial communities such as in soil, sediment, surface water, and activated sludge. Each pathway in the database is annotated with experimental metadata and kinetic information (if available) that can be used to interpret the relationship between environmental conditions and observed PFAS biotransformations.

We additionally used data from our PFAS-specific package to retrain the rule-based pathway prediction models in envPath that were previously developed using mostly non-fluorinated compounds. We show here the performance of our PFAS biotransformation pathway prediction algorithms with the likelihood that each of our studied polyfluorinated precursors will be transformed into a perfluorinated end product in a specific environment. Our database and pathway prediction system in envPath provides scientists with one organized and easily accessible online location to conveniently characterize, predict, and understand environmental PFAS contamination. This information will be essential for future efforts to remediate PFAS contaminated sites as well as for determining the source of PFAS emissions into the environment.

3.24.P-Mo285 Per- and Polyfluoroalkyl Substances (PFAS) in Ski Waxes and Snow from Cross-Country Skiing in Germany - Comparative Study of Target Analysis and Sum Parameters

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Per- and polyfluoroalkyl substances (PFAS) can enter the environment in different ways. Possible sources

are PFAS containing consumer products. One common product that makes use of the material properties of PFAS is ski wax. Here, the PFAS ensure less frictional resistance, thus allowing for increased speed. However, people who wax their skis with these waxes could absorb PFAS in their body, which can lead to health problems in the long term. Moreover, PFAS applied in ski wax abrade onto snow during use, which contaminates the environment. In our study, we analyzed various currently available ski waxes (2020s) and ski waxes from the 1980s with PFAS target analysis and with the sum parameters extractable organically bound fluorine (EOF), hydrolysable organically bound fluorine (HOF) and total fluorine (TF). Moreover, snow samples from the long-distance cross-country ski trail Kammloipe in the Ore mountains in Germany were sampled and analyzed with PFAS target analysis and the adsorbable organically bound fluorine (AOF) sum parameter to document the entry of PFAS from ski waxes into the environment. In opposite to the ski waxes from the 1980s much more ski waxes from the 2020s contain high (total) fluorine values. The highly fluorinated ski waxes contain up to approx. 6% of fluorine. The EOF and HOF sum parameters of the ski waxes are strongly decreased in comparison to the TF values (max. approx. 1000 mg/kg = 0.1%). But even the PFAS-free labeled ski waxes have EOF/HOF values in the low mg/kg range. In the snow samples from different spots of the ski trail, both the AOF sum parameter and the PFAS target analysis identified PFAS. Moreover, on a PFAS hotspot also soil samples were analyzed, which indicates that PFAS from the ski waxes adsorb after snow melting into the soil. Thus, our results show that the use of ski waxes is a possible contribution to the environmental contamination of PFAS, which is hopefully drastically reduced with the ban on fluorinated waxes by the International Ski and Snowboard Federation (FIS).

3.24.P-Mo286 Detection, Quantification, and Treatment of Per- and Polyfluoroalkyl Substances (PFAS) in Groundwater (DFEAT-PFAS)

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Per- and polyfluoroalkyl substances (PFAS) are a large group of chemicals used in the formulations of thousands of consumer goods. Because of the recent regulations and restrictions on the use of long chain (?C8) PFAS a significant shift in the industry towards short (C4-C7) and ultrashort (C1-C3) chain alternatives has been recognized in the last years. Due to the high polarity and water solubility of ultrashort PFAS, the potential for bioaccumulation is low. However, the high persistence of ultrashort-chain PFAS will result in environmental accumulation, especially in aquatic environments, leading to potential risks for aquatic organisms and increased human external exposure through drinking water. Ultrashort PFAS like trifluoroacetic acid (TFA) are low to moderately toxic to a range of organisms. In the project we are focusing on detecting and removing PFAS, especially ultrashort-chain PFAS from contaminated groundwater. Therefore, we suggest developing and optimizing short- and ultrashort-chain PFAS detection, quantification, and removal. We will design passive sampling devices, which can collect and monitor the temporal profile of PFAS species in groundwater. This will allow us to analyze PFAS contaminations in German and Israeli groundwater using state-of-the-art novel analytical techniques. In addition, contaminated groundwater will be treated via a two-stage process, designed to concentrate the relatively low PFAS concentrations by novel membrane processes including closed-circuit reverse osmosis (CCRO) and mixed matrix composite nanofiltration membranes (MMCM). Afterwards the rejected streams, containing higher concentrations of PFAS will be treated by coagulation and the remaining PFAS adsorbed onto carbonaceous nanomaterials (CNMs).

3.24.P-Mo287 Investigating the Release and Fate of Poly- and Perfluoroalkyl Substances (PFAS) in Wastewater Treatment Works by Using Diffusive Gradient in Thin-Film (DGT) Passive Samplers **Rafael Georgiou¹, Crispin Halsall² and Jonathan Barber³,** (1)Lancaster University, Lancaster, United Kingdom, (2)Lancaster University, United Kingdom, (3)Cefas, United Kingdom

Poly- and Perfluoroalkyl Substances (PFAS) are a large group of persistent and bioaccumulative synthetic chemicals found in numerous consumer products due to their thermal and chemical stability, and water- and grease-resistant properties. Their extensive applicability, detection in many environmental media, toxicity as well as ubiquitous occurrence, has led to significant regulatory responses to manage PFAS pollution by replacing legacy PFAS with novel supposedly safer alternatives. Unsurprisingly, PFAS find their way into the environment throughout their life cycle from manufacture to waste disposal.

Undoubtedly, PFAS enter wastewater and runoff streams and eventually end up in rivers and estuaries. Wastewater treatment works (WWTWs) were widely reported to be a significant source of PFAS pollution in the wider environment. Many studies have demonstrated that wastewater effluents can introduce higher PFAS amounts in the environment than influents. However, this is not always the case and WWTWs were described as sinks or secondary sources of PFAS pollution. Moreover, PFAS sources vary spatiotemporally and hundreds of fluorinated chemicals influence WWTWs emission profiles and there are still uncertainties about how PFAS are distributed in different treatment processes. Higher effluent concentrations were suggested to occur because precursor transformations during aerobic digestion can lead to the formation of terminal PFAS. Nevertheless, more studies are required to investigate the fate of PFAS during different treatment processes.

Passive sampling methods, such as diffusive gradient in thin films (DGTs) are increasingly used in studies providing time-weighted average (TWA) concentrations of organic contaminants in a period of exposure. These tools enable more accurate measurements compared to grab sampling. Using DGT samplers provide high temporal and spatial resolution that may be achieved relatively cheaply. Organisations like the U.S. EPA and frameworks such as the EU's Water Framework Directive and the Stockholm Convention have been promoting passive sampling methods for further development in future applications, such as monitoring emerging contaminants like PFAS levels in different WWTW stages.

This study aimed to utilise DGTs to examine the occurrence and fate of legacy and novel PFAS in a UK WWTW.

3.24.P-Mo288 Analysis and Monitoring of Per- and Polyfluoroalkyl Substances (PFAS) Levels in Drinking Water: Method Validation and Application in a Real Case Study

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Per- and polyfluoroalkyl substances (PFAS) are a family of industrially manufactured chemicals used worldwide in many applications, from cookware to water-repellent clothing, due to their properties. Consequently, PFAS are ubiquitously found in water resources, sediments, and sludge, with drinking water being one of the main routes of human exposure. Their C-F moiety is known as one of the strongest bonds in organic chemistry, giving PFAS their persistent behavior. As a result, some PFAS are not effectively removed in drinking water treatment plants (DWTPs) and may be present in drinking water systems. Chronic exposure to these compounds, even at trace levels, can negatively impact health, as they may act as endocrine disruptors. Accordingly, regulatory agencies have established limits for PFAS levels in tap water. In light of this, a fast and robust method is necessary for analyzing various PFAS families, with variable functional groups and analytical requirements. Therefore, a method was validated and applied to assess PFAS levels at three different water basins and in towns with individual water supply from an area with high industrial activity. Currently available analytical instrumentation, such as high-performance liquid chromatography coupled with high-resolution mass spectrometry, offers a broad scope for analyte identification, including both target and suspect analyses with high precision at trace levels. With this in mind, we validated and applied a solid-phase extraction method using Oasis HLB to measure various PFAS, including carboxylates, sulfonates, ethers, amides, and emerging species. The method's performance was tested through interlaboratory assays, yielding promising results that meet current regulatory requirements. Regarding the real case study method application, the results indicated detectable levels of PFAS at all sampling sites, as well as in DWTPs, underscoring the importance of regular studies of this nature. However, the detected concentrations were below the limits set by authorities, indicating a low risk of PFAS exposure through drinking water in the study area. Nevertheless, the estimated daily intake and hazard index calculated for some PFAS varied significantly from town to town, suggesting different exposure patterns. Similarly, it was observed that DWTPs did not adequately remove these compounds, and in some cases, levels increased after water treatment, indicating a need to review DWTP protocols.

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3.24.P-Mo289 Declining Trends of PFASs in the Pearl River Estuary, China: Insights from Oyster-Based Biomonitoring (2015-2020)

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Due to the restrictions imposed by the Stockholm Convention on perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), emerging per- and polyfluoroalkyl substances (PFASs) have been introduced globally as substitutes, while the shift patterns have been limitedly unknown. In this study, oysters were used as bio-monitors to investigate the concentration and compositional changes of PFASs in the Pearl River Estuary (PRE), China, over a period of six years from 2015 to 2020. Overall, the total concentration of PFASs in oysters from the PRE showed a sharp decrease from 2015 to 2020, indicating that these products are gradually being withdrawn from the market due to China's implementation of the Stockholm Convention. Specifically, significant reductions were observed in perfluorobutanoic acid, 1H,1H,2H,2H-perfluorooctane sulfonic acid and 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid. These compounds were the dominant PFASs present in the oysters, with their concentrations decreasing from 12.5 ± 9.10 to 0.63 ± 0.25 ng/g dry wt., from 23.4 ± 35.9 to 0.11 ± 0.08 ng/g dry wt., and from 10.3 ± 3.31 ng/g dry wt. to not detected, respectively. Higher concentrations of emerging, than legacy, PFASs likely reflect their higher bioaccumulation potential and more introduction. Continued PFASs use will inevitably lead to increased environmental and human exposure if not controlled. Additionally, oysters exhibited higher PFASs loads during the wet season compared to the dry season, indicating that the unique climate and environmental factors in the PRE influenced the environmental behavior of PFASs. Our study underscores the utility of oysters as sentinels for evaluating PFASs regulation success and highlights the value of continued monitoring.

3.24.P-Mo290 Leaching of PFAS from Soil to Groundwater in the Netherlands: Potential Implications and Measures for Dutch Drinking Water

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Approximately 60% of Dutch drinking water is sourced from groundwater. While it is well known that PFAS are ubiquitously present in media such as soil and water, less is known about their current spatial depth distribution and their time-dependent migration within and between these two media. Preliminary calculations suggest that a significant portion of the PFAS load resulting from historical and current deposition is present in the unsaturated zone of the subsurface. The mobile characteristics of PFAS raises the question when and to what extent this historical PFAS load might reach deeper groundwater and potentially lead to future contamination of Dutch drinking water reserves.

With this study we therefore aim to obtain insight of the distribution of PFAS loads in soil and groundwater over depth in drinking water protection areas in the Netherlands. We also aim to improve predictions of concentration variabilities in the topsoil, groundwater and raw drinking water, leaching from soil to groundwater and transport from groundwater to drinking water reserves. The third aim is to develop and evaluate realistic scenarios that take into account the costs and effectiveness of measures to reduce PFAS exposure in drinking water.

To achieve this, levels of at least 34 PFASs will be determined at various depths in soil and groundwater. Using these levels, the transport of PFAS through soil and groundwater will be modelled and its results will be extrapolated for different types of groundwater extractions. Finally, an estimation will be made of the influence of representative measures on the generic prediction of the PFAS concentration and its associated exposure. This knowledge enables governments and the drinking water sector to make better-informed decisions regarding potential measures.

3.24.P-Mo291 Monitoring of Per- and Polyfluoroalkyl Substances (PFAS) in German Wolves (*Canis lupus*)

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Per- and polyfluoroalkyl substances (PFAS) are a group of organic chemicals of solely anthropogenic origin. Due to several desirable properties, they are used in many industrial and commercial applications. Because of their persistent nature, PFAS are also known as forever chemicals and once they enter the

environment they can accumulate in wildlife, especially in scavenger and predator species.

The detection of PFAS and their impact on wildlife as well as the detection of emerging emission sites still challenges researchers and government agencies. The presented study aimed to measure the PFAS burden in liver samples ($n = 150$) of adult wolves (*Canis lupus*) from four German federal states (Brandenburg, Saxony, Saxony-Anhalt and Lower Saxony) covering a time span of ten years (2013 to 2023) representing years of return and establishment of the species to growth of the population in these areas. To achieve this, a target method for the quantification of 30 PFAS via ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) was used.

PFAS levels above the limit of quantification (LOQ; LOQs between 0.05 and 10 $\mu\text{g/kg}$) were found in all liver samples. Total PFAS concentrations ranged from 2.1 to 363 $\mu\text{g/kg}$ wet weight. The predominant substance was perfluorooctane sulfonic acid (PFOS) representing between 7% and 100% of the samples total PFAS burden, followed by medium-chain to long-chain perfluoroalkyl carboxylic acids (PFCAs from C7 to C13) and perfluoroalkyl sulfonic acids (PFSAs; C6, C7 and C10). Besides PFCAs and PFSAs, two additional PFAS (ADONA and 6:2 fluorotelomer sulfonic acid (6:2 FTS)) were detected in some samples. Over the course of ten years, no statistically relevant change in PFAS concentrations (assessed for PFOS, perfluorooctanoic acid (PFOA) and the sum of all PFAS) was observed. Furthermore, there was no difference in PFAS concentration between female and male wolves. A spatial comparison on the federal-state level showed large deviations within the four surveyed German states, but a closer look showed individual PFAS hotspots close to possible emission centers such as industrial parks and mining areas. Therefore, the results of this study show that the PFAS burden of adult wolves' liver might be indicative of the position of emerging and existing emission centers.

3.24.P-Mo292 The Role of Sea Spray Aerosols in the Atmospheric Transport of Perfluoroalkyl Acids to the Arctic

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Perfluoroalkyl acids (PFAAs) are highly persistent environmental pollutants that have become ubiquitously distributed after decades of production and use. However, some uncertainties remain regarding their long-range atmospheric transport to the Arctic. Notably, PFAAs in seawater can be effectively enriched in sea spray aerosols (SSA) and remobilized from the ocean to the atmosphere. SSA have already been shown to be an important contributor to PFAA concentrations in coastal Arctic air. A previous study from our group demonstrated significant correlations between PFAA concentrations in air samples collected at Andøya, Norway, and levels of SSA tracer ions. More recently, such correlations were also observed in ice core samples from northern Ellesmere Island, Nunavut, Canada, implying that SSA may serve as a relevant transport mechanism for PFAAs in the High Arctic.

To further improve our understanding of the role of SSA as a source of PFAAs in the Arctic, this present study collected aerosol samples from the Canadian High Arctic station of Alert, Nunavut, Canada. The station is located at the northeastern tip of the Ellesmere Island and approximately 47 km from water which is covered by sea ice for most of the year. The samples were analyzed for PFAAs and SSA tracer ions, including sodium (Na^+) and magnesium (Mg^{2+}). Correlation analysis and air mass trajectory analysis were conducted to evaluate the contribution of SSA to atmospheric PFAA levels in the Arctic region.

3.24.P-Mo293 The INBO Fish Tissue Collection: A Historical Resource

Lies Teunen, Emily Veltjen, Marieke Desender and Caroline Geeraerts, Research Institute for Nature and Forest, Belgium

The Research Institute for Nature and Forest (INBO) is an independent research institute of the Flemish Government that collects valuable Natural Science objects which can be used for applied scientific biodiversity research. A selection of these objects is archived as the INBO collection for the purposes of validation and reuse. This collection is currently divided in eight subcollections according to specific research activity, workflows and research teams. The INBO Fish Tissue Collection is one of the INBO subcollections that contains frozen (-20°C) freshwater fish samples (mainly muscle tissue of European eel *Anguilla anguilla*) collected since 2000. For each specimen locality (X,Y coordinates and date), species, length and weight is documented. This collection contains, amongst other potential data, an enormous source of information on historical pollution. Therefore, it can be used as a screening tool for the Flemish water bodies or for temporal trend monitoring in follow-up of remediation or clean-up actions. This allows for the identification of pollution hotspots in the past and can serve as a baseline for more in depth investigation of potentially contaminated areas. Previously, the INBO Fish Tissue Collection has been successfully implemented to study PFAS in 'historical' eel samples. Subsequent time trend analysis shows

decreasing trends in PFAS concentrations for multiple compounds. Furthermore, locations of high concern could be identified. In the coming years, there are plans to better integrate this collection with other data sources and ensure it adheres to the FAIR principles (Findable, Accessible, Interoperable and Reusable) by connecting it to the DiSSCo (Distributed System of Scientific Collections) (Flanders) infrastructure. The collection itself will be published on GrSciColl and the institutional website to increase visibility, while the specimen data will be published on DiSSCo. The main aim is to promote use of, and collaboration with, the INBO Fish Tissue Collection to accelerate progress in pollution research. We encourage researchers not to wait until the data is published to start a collaboration.

3.24.P-Mo294 Looking Back on PFAS Contamination in ‘Historical’ Eel Samples From Flanders, Belgium (2000-2008) and the Comparison to More Recent Results

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The technical progress in chemical analysis enables monitoring of the pollution load more accurately, including the group of per- and polyfluoroalkyl substances (PFAS). In order to evaluate time trends, a glimpse into the past can provide valuable information. The INBO Tissue Collection contains fish samples (mainly eel muscle), collected and preserved (-20°C) since 1994 and allows for this comparison. In the current study, eel muscle tissue samples, collected between 2000 and 2008, were selected from 28 sampling sites. In total, 42 PFAS components were analysed on these samples. In this way, the distribution and magnitude of historical contamination could be mapped, and the identification of potential hotspots in the past was made possible. The total sum of PFAS was 102 ± 74 ng/g ww and PFAS profiles were mainly dominated by PFOS, PFBS and 8:2 diPAP. A few hotspots were identified with elevated concentrations of PFOS (188 ng/g ww), PFBS (183 ng/g ww), 8:2 diPAP (209 ng/g ww) and a single high reading of 6:2 FTS (31 ng/g ww). The ecological and human health risks were estimated for the historical concentrations. At 75% of the sites, the environmental quality standard for PFOS in biota was exceeded (9.1 µg/kg ww). In addition, at 32% of the sites, an exceedance of the EFSA consumption standard was measured (sum of PFOS, PFOA, PFNA and PFHxS), corresponding to a weekly allowable amount of less than 8 g (long-term exposure). Time trend analyses revealed that nowadays average PFOS concentrations were reduced with 72%. In general also concentrations of other components such as PFBS, 6:2 FTS, PFOA and PFNA reduced. However, locations with high PFAS concentrations are still present. Besides, for some components having high historical concentrations, such as 8:2 diPAP, a time trend analysis was not feasible due to variable high detection limits. This emphasises the need to further investigate this component accurately in future monitoring, using more accurate analytical methods present to date. Overall, this study underlines the importance of performing analyses on such historical samples to identify and continue to monitor problem areas. The current study can therefore be seen as a first-line screening to identify areas that need further investigation to assess pollution issues. Finally, historical data also provides a better picture of the evolution of pollution and the efficiency of certain restrictions or treatments to reduce pollution pressure.

3.24.P-Mo295 PFAS Pollution in Fish and Water from the United Kingdom and Spain: Temporal Trends and Implications for Human Dietary Exposure

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Poly- and perfluoroalkyl substances (PFAS), widely used since the 1940s for their water- and oil-repellent properties, persist in the environment, accumulating in the food chain and raising health concerns. This study examines 46 PFAS in 238 fish samples from the UK and Spain, including muscle fillet and whole fish from 16 species. Complementary data on water from the UK Environment Agency (2000-2024) provide additional context. Analytical methods involved LC-HRMS for target and suspect PFAS screening.

Results show higher PFAS concentrations in Spanish fish, likely due to the semi-closed system of the Mediterranean Sea. Key findings include the presence of ultra-short-chain PFAS like TFA and TFSA in 25% of samples. While median dietary intakes of PFAS through fish consumption in the UK and Spain are below EFSA's thresholds, mean values exceed limits, highlighting health risks. Species differences suggest bioaccumulation, with fish like Coley, Sea Bass, Mackerel, and Hake posing higher risks.

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3.24.P-Mo296 Quantifying PFAS Release from Solar Panels During the Use Phase

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Solar technology plays a pivotal role in the current green transition and produced >1250 TWh globally in 2022. While PFAS-free alternatives and technologies exist to ensure durability their use only made up approximately 20% of the market share in 2022. Solar panel producers widely claim PFAS free panels but apply analytical methods with detection limits ranging from 0.01-0.1 mg/kg, which is in stark contrast to current environmental quality standards (EQS) for e.g. surface waters in the sub µg/L range.

Consequently, novel methods are needed for up-stream quantification of the potential release during the use phase with detection limits reflecting the current EQS.

The aim of this study was to develop a screening method utilizing wiping with a solvent for quantification of potential PFAS release during the use phase. The method was applied to eight different solar panels and the release was compared to the presence of PFAS in environmental background samples in the study area. The estimated releases were also used to calculate the contaminant mass discharge and resulting EQS for a case-study area.

All samples were measured for 20 PFAS (PFPeA, PFBS, PFHxA, PFPS, PFHpA, PFHxS, 6.2 FTS, PFOA, PFHpS, PFNA, PFOS, PFNS, PFDA, PFDS, PFUdA, PFOSA, PFDoA, PFDoDS, PFTTrDA and PFTTeDA). The quantification limit of the method was 0.1 ng/wipe with an average recovery of the extraction method for the tested PFAS of 91±8% using standard addition with mixed PFAS standard of 10 ng/wipe. Indoor backgrounds were below quantification limits for all PFAS analyzed. The outdoor backgrounds for natural deposition resulted in the first 2 wipes exceeding the quantification limit for all measured PFAS except PFOA and PFOSA with an average sum of the remaining PFAS of 2.3 ng/wipe. The third wipe of the outdoor environmental background resulted in none of the measured PFAS above the quantification limit. Among the tested PFAS, only PFHxA (perfluorohexanoic acid) was found above the quantification limit in 6 out of 8 solar panels. The release of PFHxA ranged from 4.3-83 ng/m² solar panel, with a corresponding contaminant mass discharge of the case study area of 2.7 52 mg during the use phase of the solar panel. Assuming direct transport to the groundwater the resulting concentration for a total release within 1 year would be 800-1400 times lower than the current EQS of 100 ng/L.

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3.24.P-Mo297 Spatial and Seasonal Characterization of 17 PFAS in Two Different Estuarine Systems Along the Portuguese Coast

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Per- and polyfluoroalkyl substances (PFAS) have been produced and used in industrial and commercial products for more than 6 decades. Attending to their chemical stability, bioaccumulation potential, high persistence in the environment and toxic effects are a topic of great concern. The main goal of this study was to do a spatial and seasonal characterisation of 17 different PFAS in estuarine waters and organisms (e.g. crustacean *Carcinus maenas*) in two Portuguese estuaries (Douro and Tagus), with a high industrial load). These two different estuaries had similar ?PFAS concentrations along the time. In general, for the Tagus estuary, winter (2.33-10.18 ng/L) and spring (4.49-8.28 ng/L) presented the highest ?PFAS concentrations compared to summer (1.93-4.17 ng/L) and autumn (0.06-1.15 ng/L), except for Trancão (Tagus) in summer with a total of 25.67 ng/L. PFOA and PFOS were the most relevant compounds in our sampling sites. For the Douro estuary, the ?PFAS concentration was similar between seasons, with values ranging from 0.98-18.68 ng/L, 3.77-25.07 ng/L, 0.90-7.51 ng/L and 4.82-12.83 ng/L observed in autumn, winter, spring and summer, respectively. The diversity of compounds found was higher in the Douro (n = 17) than in Tagus (n = 6), being the most relevant ones the PFOA, PFOS and PFBS. Regarding the biological matrices, preliminary results for the crustacean *Carcinus maenas*, for the Tagus estuary (e.g. Seixal) indicated higher PFAS levels in the autumn and summer comparatively to the remaining seasons.

A pattern can be observed between the seasons with regard to the prevalence of different PFAS. In summer, shorter-chain PFAS (PFHpA and PFOA) are the most common, while in winter, longer-chain PFAS (PFDoA, PFTrDA, PFTeDA, 9Cl PF3ONS and 11Cl-PF3OUdS) are most predominant. Despite the PFAS concentrations in surface waters were below the Environmental Quality Standards (PFOS = 36000 ng/L), it is relevant to continue to monitor the coastal areas and evaluate possible impacts in the trophic web.

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3.24.P-Mo298 Evaluating the Adsorption Performance of Activated Carbon for In-Situ Remediation at PFAS Contaminated Sites

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Due to their extreme persistence, often combined with high aqueous mobility, per- and polyfluoroalkyl substances (PFAS) are challenging to remove from the environment. The in-situ application of (colloidal) activated carbon ((C)AC) aims at a barrier effect against the further spreading of PFAS in the aquifer. This requires high-performance activated carbon materials that ensure high retardation of PFAS at low loading on the aquifer sediment (0.1-1 wt%) and thus long lifetimes in the order of decades.

Here, we will discuss the development and testing of injectable CAC for in-situ sorption barriers. Our studies include a commercially available CAC, Intraplex® (Intrapore GmbH, Germany), and an AC at the research stage, Activated Carbon Spheres (ACS). ACS are produced by bottom-up synthesis from sucrose, a locally available, renewable raw material, using hydrothermal carbonization and subsequent activation with chemical agents or gas activation with water vapour (Balda et al. 2023). This route allows the production of high-purity spherical carbon particles with a mean diameter of around 1 µm and a microporous structure. The mean pore diameters as well as the surface chemistry of the ACS can be specifically adapted to the respective application by varying the activation parameters. In comparison, commercial CAC is typically produced by top-down synthesis using wet-grinding of granular AC produced from raw materials such as lignite or hard coal.

The study presents the optimisation of the ACS material for the adsorption of perfluorooctanoic acid (PFOA), a particularly relevant representative of the PFAS. The focus was on tailoring the pore system and the surface chemistry in order to achieve optimum adsorption performance and high ageing resistance of the material. These material properties are crucial for the application of the adsorbent in an in-situ sorption barrier. Furthermore, the retardation of various PFAS by immobilized ACS from column tests are presented. These results were used to predict the long-term performance and lifetime of in-situ barriers in contaminated aquifers.

3.24.P-Mo299 In-Depth Per- and Polyfluoroalkyl Substances (PFAS) Assessment in Marine Organisms from the Seine Estuary (France) using Suspect and Non-Target Approaches

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Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals known for their unique properties, which have led to widespread environmental contamination, particularly in marine ecosystems. While some legacy PFAS have been regulated, emerging alternatives complicate contamination profiles. High-resolution mass spectrometry (HRMS), coupled with non-target and suspect screening, provides a broader perspective on PFAS contamination. However, these methods have primarily been applied to heavily

contaminated sites and top predators, with limited use in lower trophic organisms. Yet, such studies are essential for detecting PFAS precursors that may undergo biotransformation at higher trophic levels. This study aims to explore the potential of non-target screening to provide a comprehensive overview of PFAS contamination, including precursors, across various trophic levels. Marine species, ranging from shellfish to fish, were collected from the Seine Estuary, English Channel, France, in 2021 and analyzed using both liquid and gas chromatography-HRMS. Composite solutions of electrochemical fluorination (ECF)-based aqueous film-forming foams (AFFFs) and telomerization (FT)-based AFFFs were used as reference materials to support detection and identification. Suspect and non-target screening was conducted using EI-MAVEN and PF?Screen software. Over 50 PFAS from 21 classes were detected, including non-biotransformed precursors, some of which were identified for the first time in marine organisms, such as 6:2 fluorotelomer sulfonyl amido sulfonic acid (6:2 FTSO?AoS). Perfluoroalkyl compounds were predominantly characterized by 8 perfluorinated carbons and detected in ECF AFFFs, while polyfluoroalkyl compounds were primarily 6:2 fluorotelomer structures, which occur in FT AFFFs. Given that more than 60% of the identified PFAS are typical of AFFFs suggests that AFFFs are a major contamination pathway. Ongoing GC-HRMS analysis will complement the LC data by detecting neutral PFAS. This study provides a detailed overview of PFAS contamination in marine organisms from the Seine Estuary, revealing significant pollution with largely unregulated compounds. The combined analysis of low trophic level species and AFFF references proved effective for identifying non-biotransformed precursors. This work highlights the value of integrating LC- and GC-HRMS for comprehensive PFAS analysis and identifies key compounds for future regulatory and monitoring efforts.

3.24.P-Mo300 Assessment of PFAS Adsorption in PBAT Bio-Based Microplastics

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Per- and polyfluoroalkyl substances (PFAS) are a class of persistent organic pollutants that have raised significant environmental and health concerns due to their widespread contamination and resistance to degradation. Despite extensive research on their environmental presence, little is known about their interactions with bio-based microplastics which may act as vectors for PFAS transport and accumulation in terrestrial environments. Polybutylene adipate-co-terephthalate (PBAT), a biodegradable plastic, has gained attention as an environmentally friendly alternative to conventional petroleum-based plastics, especially when blended with inulin for use in packaging. This study investigates the adsorption of four PFAS (HFBA, PFOA, FOSA and PFOS) on pure and inulin-blended PBAT (100-200 µm) over 56 days. Following HPLC-MS-MS analysis, the results revealed distinct adsorption patterns for each PFAS over time. Regarding adsorption on pure PBAT, HFBA showed initial adsorption at 0.5 hours, but no further adsorption was observed until 8 hours. After that, it increased and peaked at 2 days before returning to 0% at 14 days. PFOA only exhibited adsorption at 14 days. In contrast, FOSA showed the highest adsorption, starting at 38.71% at 8 hours and reaching a maximum of 93.05% at 14 days. These findings showed that FOSA exhibits the most significant and sustained adsorption on pure PBAT or inulin-PBAT. While HFBA and PFOA show delayed and lower adsorption patterns. Overall, the study provides insights into the interactions of PFAS with PBAT bio-based microplastics and emphasises the potential of PBAT as a component of PFAS accumulation in the environment.

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3.24.P-Mo301 Isolation of PFAS-Degrading Bacteria and Assessment of Microbial Diversity in Contaminated Soil in North Italy

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The increasing contamination of soil and groundwater with per- and polyfluoroalkyl substances (PFAS) presents significant environmental challenges due to their persistence, mobility, and their hazardous effects. The high C-F bond strength makes PFAS non-biodegradable in the environment, and despite some in vitro evidence, their biodegradation in the environment remains an ongoing challenge. The presented research aims to address this issue through two main objectives: (1) isolating and identifying promising PFAS-degrading microorganisms from contaminated sites, and (2) assessing the microbial diversity in PFAS in soils sampled in PFAS polluted areas of Northern Italy. The first objective involves the application of sequential enrichment approach targeting two PFAS compounds (PFOA and HFBA), followed by molecular and non-molecular microbiological techniques, to identify and characterize microbial strains with biodegradation potential. The second objective was the analysis of the microbiome of soil, water and plants sampled from heavily contaminated sites of the Veneto Region. High-throughput metabarcoding was employed to characterize microbial diversity, providing insight into the potential for PFAS bioremediation. Additionally, enzymatic activity expressed by the microbial communities were assessed to understand their role in the degradation of PFAS. This research will provide new insight into PFAS degradation and could contribute to the development of sustainable bioremediation strategies for PFAS contaminated environments.

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3.24.P-Mo302 Expanding the Search for PFAS in San Francisco Bay: Emerging Trends in Surface Water and Sediment

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Per- and polyfluoroalkyl substances (PFAS) are a broad class of compounds that are used and found ubiquitously in consumer and industrial products. Their high persistence, bioaccumulation potential, and toxicity have increasingly raised concerns of their adverse impacts on human and ecological health. In the San Francisco Bay, a highly urbanized, enclosed estuary in California, USA, PFAS have been detected throughout the environment since monitoring began in 2006. However, early analyses generally only included 13 individual PFAS via targeted methods. Building on these previous efforts, this study examined Bay surface water and sediment with an expanded analyte list that includes 27 additional PFAS (using EPA Method 1633) as well as the total oxidizable precursors (TOP) assay, which indirectly quantifies oxidizable PFAS precursors by conversion to terminal perfluorinated carboxylates (PFCAs), to assess the level of unknown precursors not detected using traditional approaches.

Overall, individual PFAS were detected throughout the Bay, notably in sites in the southern portions of the Bay where previous high detections in the environment have been found. In particular, perfluorooctane sulfonic acid (PFOS) was predominantly found in sediment, with statistically significant declines in South Bay levels throughout four sampling efforts from 2012 to 2023. In contrast, results from the TOP assay suggested the presence of unidentified precursors in sites located throughout the Bay, not just in the southern region. Summed TOP assay concentrations were up to 8x and 20x greater in water and sediment samples, respectively, when compared to targeted analysis of the same sample. These findings indicate broad, non-targeted analyses are needed to fully understand the scope of PFAS contamination, and additional PFAS, especially known precursor compounds, should be included in risk evaluations.

3.24.P-Mo303 Estimation of Background Contamination with Per- and Polyfluorinated Substances (PFAS) in Various Environmental Matrices by Measuring Plants, Soil and Water Samples From Two National Parks in Austria

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Today we know that PFAS are found ubiquitously, due to their wide range of applications. Major sources for PFAS in the environment are the chemical industry, wastewater treatment plants (WWTP) and waste incineration facilities (WIP), firefighting foams and the agricultural application of biosolids as well as landfills and their leachates. The maximum tolerable amount of PFAS concentration in soil and water is becoming regulated in different regulations and the actual limit values will probably continue to decrease. The aim of this work was to evaluate given background contamination in different environmental

matrices, as limit values are always restricted by background concentrations. To investigate PFAS background levels in Austria several environmental samples, including water, sediment, soil and wild plants were collected in the national parks Donauauen and Neusiedlersee.

The analysis was done for the 20 PFAS regulated in the European Drinking Water Directive. Various perfluorocarboxylic acids (PFCAs), PFOS and the presence of precursors were detected in the water samples in the range of 0.2-14 ng/L at all sites. In the sediment samples PFAS could be hardly detected at concentrations above 25 ng/kg dry weight (dw). The soil samples dominantly contained PFCAs and PFOS at concentrations up to 100 ng/kg dw. Across all plant samples the short-chain PFBA showed the highest concentrations with a mean of around 1300 ng/kg dw and accounting for more than 75 % of the sum of 20 EU-PFAS. The long-chain PFOS showed a mean concentration of more than 60 ng/kg dw followed by PFHpA, PFNA and PFOA.

As expected, PFAS can be detected in every environmental matrix and accumulation is highest for short-chain PFAS, especially PFBA, in plant samples. The spot with the highest detected PFAS concentration in the water and plant samples was Strandbad Illmitz in the national park Neusiedlersee. The enhanced concentrations of PFAS at the lido clearly show the anthropogenic influence on PFAS input into the environment.

With reference to the current limit values in the Austrian regulations for drinking water (TWW 2024) and soil (BAWP 2023), the assessed background contamination in the corresponding matrices is significantly lower. Referring to the Austrian QZV Chemie for surface water, in which the good chemical status is defined, the annual average environmental quality standard for PFOS with 0.65 ng/l could even be exceeded in national parks.

3.24.P-Mo304 Validation of a Method for Assessing Per- and Polyfluoroalkyl Substances (PFAS) in Human Serum from South Florida (USA) Populations Using Online SPE-LC-MS/MS

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Per- and polyfluoroalkyl substances (PFAS) are man-made chemicals linked to health risks including cancer, and environmental persistence. Their widespread use raises concerns about human exposure, with drinking water being one of the most common routes. This study validates a method to measure PFAS in human serum using online Solid Phase Extraction combined with Liquid Chromatography-Tandem Mass Spectrometry (SPE-LC-MS/MS). The method is validated to analyze serum samples from individuals with liver cancer and matched non-cancer controls in South Florida, selected from Miami-Dade County zip codes based on liver cancer mortality counts.

The method validation involves optimizing SPE conditions, chromatographic separation, and mass spectrometry parameters to ensure sensitivity and reliability. It evaluates detection and quantification limits, sensitivity, accuracy, precision, and the effects of the serum matrix on analytical measurements. Stable isotope-labeled standards are used to mitigate matrix effects and enhance accuracy.

Serum samples for this study are provided in collaboration with a local South Florida Biospecimen Repository Facility. Cases and controls are selected based on zip code residency and matched for factors such as body mass index, age, gender, smoking history, ethnicity, and other relevant but limited health data. These samples align with previously analyzed drinking water data from the same areas conducted by our research group. This study provides insights into PFAS exposure patterns and concentrations in impacted communities. However, due to the limited cohort size, it is not intended to establish a direct correlation between PFAS exposure through drinking water and bioaccumulation in human serum. Future research investigating these correlations in further detail can benefit from these findings.

This study contributes to our understanding of PFAS in human serum by determining detection frequency and concentration ranges and examining patterns of bioaccumulation across different PFAS classes as well as short- and long-chain PFAS. These findings provide a foundation for addressing public health concerns related to PFAS exposure in South Florida populations and potential links to liver cancer.

3.24.P-Mo305 Assessment of PFAS Contamination in German Soils: Non-Target Identification, Total Oxidizable Precursor Assay and Microcosm Studies

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The Rastatt case highlights a significant instance of agricultural topsoil contamination with per- and polyfluoroalkyl substances (PFAS) in southwest Germany. This contamination originated from the

application of paper sludge in topsoil. In this study, 40 PFAS were monitored in eight topsoil samples from the Rastatt/Baden-Baden and Mannheim regions, following the EPA 1633 guidelines. In addition to targeted PFAS analysis, non-target screening was performed to identify PFAS precursors. The screening revealed phosphorus-containing PFAS precursors, including several homologues of FTMAPs and diPAPs, as well as diSAmPAP, which collectively accounted for over 80% of the total PFAS burden in the soil. Nonetheless, some overlooked precursors may still be present in some of the monitored locations. The identification of specific PFAS intermediates, along with previous findings of perfluoroalkyl acids (PFAAs) in groundwater, suggests that biotransformation of precursors occurred in the topsoil, followed by the leaching of PFAAs into groundwater. To further investigate the factors influencing biodegradation and to estimate potential contamination time scales, the study combined chemical oxidation of precursors (TOP assay) with extractable organic fluorine (EOF) measurements to evaluate the PFAS reservoir in the soil.

Batch microcosm incubations were also conducted to monitor PFAA concentrations over time. The observed increase in PFAA concentrations was attributed to the breakdown of PFAS precursors. Based on these findings, PFAA generation rates were estimated. To better understand the variability in degradation rates across the eight different soils, the role of microbial biomass carbon and phospho(mono/di)esterase enzymatic activities was analyzed using principal component analysis (PCA). This approach explained the differences in observed rate constants.

The study also assessed contamination time scales, which indicated that the transformation of precursors and subsequent leaching of short-chain PFAS would likely persist for decades. The findings underscore the utility of combining non-target screening, TOP assay, and EOF approaches for environmental PFAS analysis. Moreover, the study provides a methodology to estimate contamination time scales in cases of mixed PFAS pollution. Our results also suggest that microbial biomass carbon and specific phospho(mono/di)esterase enzymes may play a role in PFAS biotransformation, warranting further investigation.

3.24.P-Mo306 Impacts of Sea Spray Aerosol Transport of Perfluoroalkyl Acids (PFAAs) on Coastal Regions

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Coastal contamination by perfluoroalkyl acids (PFAAs) has emerged as a pressing environmental and public health concern. In Denmark, elevated concentrations of PFAAs in environmental media such as groundwater, soil and vegetation have been detected on the northwest coast of Jutland despite the absence of obvious local sources. These concentrations decline sharply within a few kilometers inland, implying that the atmospheric transport of PFAAs via sea spray aerosols (SSA) from the Northern Sea may be a significant source. Previous laboratory and field studies have shown that PFAAs are strongly enriched in SSA, and large SSA particles (>10 µm) are likely driving substantial dry deposition near coastal zones, where they pose risks to groundwater and drinking water supplies.

This project aims to unravel the processes governing inland PFAA transport and deposition from coastal areas, focusing on SSA-mediated mechanisms. We will conduct air sampling and atmospheric dry/wet deposition measurements along the Danish coast to investigate the direct evidence of SSA-driven PFAA contamination in these regions. By integrating field measurements and high-resolution numerical modeling, we will evaluate the impacts of SSA-mediated atmospheric transport of PFAA on their concentrations in coastal and inland areas.

The findings from this research will advance understanding of PFAA dispersion in coastal environments, improve predictions of contamination risks and inform regulatory and mitigation strategies. This work carries critical implications for safeguarding coastal drinking water resources and addressing the persistent global challenge of PFAS pollution.

3.24.P-Mo308 Determination of Persistent and Mobile Organic Compounds in the River–Groundwater Interface of the Besòs River Delta, Spain, Using a Wide Extraction Approach

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Climate change impacts the Mediterranean region, transforming it from region with a semi-arid climate to

a region with an arid climate. Under this situation, while groundwater is an essential hydric resource, its existence is in danger due to anthropogenic pressures. Persistent mobile organic compounds (PMOCs) have recently been recognised as an emerging problem; however, PMOCs in groundwater need to be better characterised. Here, we present a new analytical method to characterise the profile of PMOCs in groundwater based on two parallel solid-phase extraction (SPE), using weak anion exchange and weak cation exchange. Extracts were analysed by ultraperformance liquid chromatography (UPLC) using mix-mode chromatography for those compounds analysed under negative ionisation conditions and hydrophilic interaction liquid chromatography (HILIC) under positive conditions coupled to high-resolution mass spectrometry (HRMS) using a Q-Exactive Orbitrap analyser. For the suspect screening of PMOCs in groundwater, the acquisition mode was in full scan (FS) by independent scan of all ion fragmentation. For the tentative identification, different online databases such as Environmental and Food Safety (EFS) HRAM Compound database, PFAS NIST database, ChemSpider for chemical structural information, MzCloud as a mass spectral database, and an in-house list with 1280 PMOC structures have been used. The performance of the method was assessed with 29 representative PMOCs which were selected based on the previous literature. The recovery rates have been between 63-110% for 90% of the target compounds and method limits of quantification (MLQ) between 0.3 and 10.5 ng/L.

The optimised approach was applied to assess PMOCs in the Besòs River aquifer, NE Spain, showing 148 tentatively identified structures at confidence levels 1-3. Among them, 66 suspects were tentatively identified at level 3, 54 at level 2, and 28 confirmed at level 1. Most of these compounds were polar and highly polar compounds which are difficult to retain with other extraction approaches. Major detected compounds were pharmaceuticals and personal care products (46), followed by perfluoroalkyl and polyfluoroalkyl substances (PFAS) (32), industrial additives (27), and pesticides (23), among other groups. Some compounds, such as ultrashort chain PFAS and fluorinated betaines, were detected for the first time in groundwaters in Spain.

3.24.P-Mo309 PFAS in the Air: Insights from the Belgian Frontline

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The PFAS crisis in Belgium, triggered by roadworks near a PFAS production plant, necessitated urgent analytical solutions. The Flemish Environmental Reference Laboratory at VITO rapidly developed advanced methods to meet this demand, going beyond state-of-the-art approaches to address diverse environmental matrices, including flue gases and ambient (outdoor) air. These efforts combined target analysis (LC-MS/MS) with non-target analysis (NTA) using high-resolution MS, expanding the detection scope to PFAS compounds, classes, and precursors.

In flue gases, a validated method (LUC/VI/003) quantified 28 PFAS compounds with measurement uncertainties below 50%. Sulfonic acids were captured early in the sampling train, while carboxylic acids and short-chain PFAS were found in downstream fractions. The method highlighted challenges, such as glassware contamination by long-chain PFAS, emphasizing the importance of rigorous cleaning protocols. For outdoor air, PFAS quantification was achieved using active (filters and PUF/XAD) and passive (deposition jars) sampling. Less volatile compounds (e.g., PFOA, PFOS) were retained in filters, while volatile compounds (e.g., MePFBSA) were captured in PUF/XAD. Initial findings revealed high levels of ultra-short-chain PFAS (e.g., TFA, PFPrA, PFPrS) in PUF/XAD, prompting ongoing validation of sampling and analytical methods. Method optimization underscored the critical role of matching mass-labeled internal standards for accurate quantification.

NTA of selected samples uncovered novel PFAS classes (e.g., H-substituted carboxylic acids) and expanded known ones (e.g., PFHpSA), with unknown compounds representing up to 50% of detected PFAS. These findings highlight NTA's value in uncovering previously unmonitored PFAS.

This work advances PFAS monitoring by providing robust methodologies tailored to diverse matrices. Sharing challenges and solutions encountered during method development fosters collaboration and enhances the scientific community's ability to respond to emerging environmental crises.

3.24.P-Mo310 Distribution and Characteristics of Per- and Polyfluoroalkyl Substances (PFASs) in Surface Waters of the Western Pacific Ocean

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Per- and polyfluoroalkyl substances (PFASs) are widespread in marine environments, posing potential ecological risks to aquatic organisms and humans. However, knowledge of their transport mechanisms and ultimate fate in open oceans remains limited. This study investigates the contamination status of PFASs in surface seawater across the western Pacific Ocean and adjacent marginal seas, including the Taiwan Strait, East China Sea, and the Kuroshio Current. Six of the eight target PFASs were detected, with total concentrations of eight PFASs (8 PFASs) ranging from below detection limits to 5.09 ng/L. Elevated PFAS concentrations were observed in the East China Sea, with levels significantly decreasing from river mouths to offshore areas, indicating riverine input as a major source of anthropogenic pollutants. Notably, PFASs were also identified in the Kuroshio Current, suggesting contamination in its typically ultraoligotrophic and pristine waters. Across the study region, perfluorobutanesulfonic acid (PFBS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) were the predominant PFASs. These findings provide critical insights into the distribution and behavior of PFASs in global open ocean environments.

3.24.P-Mo311 Advanced Modeling of PFAS Sorption in Soils Through Machine Learning

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Emerging and novel per- and polyfluoroalkyl substances (PFASs) present a significant challenge due to their environmental persistence, unique physicochemical properties, and widespread use across industries. While efforts to phase out legacy PFASs progress, innovative tools are urgently needed to understand the behavior of newer compounds and inform risk management strategies. This study introduces PFASorptionML, a machine learning tool designed to predict the sorption behavior of PFASs in soils, offering insights into their environmental mobility and potential risks. Using a dataset encompassing 51 PFAS compounds across 455 soil and sediment samples, the model integrates compound-specific parameters, including molecular weight, hydrophobicity, and pKa, with soil properties like pH, organic carbon content, texture, and cation exchange capacity. For the first time, PFAS speciation is explicitly addressed in sorption predictions. The tool's sensitivity analysis reveals the dominant role of hydrophobic interactions in PFAS sorption, emphasizing the importance of compound structure in environmental behavior. Beyond accurate predictions, PFASorptionML enables the generation of high-resolution mobility maps by linking with soil data repositories, providing a valuable platform for environmental risk assessments and supporting the transition to safe and sustainable PFAS alternatives.

3.25 Emerging and Novel Per- and Polyfluoroalkyl Substances (PFASs): Latest Findings and Innovation Towards Safe and Sustainable Alternatives

3.25.T-01 Colloidal Side-Chain Fluorinated Polymer Nanoparticles are a Significant Source of PFAS Contamination in Textile Wastewater

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Side-chain fluorinated polymers (SCFP) based on per-and-polyfluorinated substances (PFAS) are used to create water-repellant and stain-resistant fabrics on textiles. The discovery of unexplained C4 C8 perfluorocarboxylic acids (PFCAs) at concentrations ranging from 50-1,000 ng/L in public drinking water supplies downstream from textile industry discharges in North Carolina initiated an investigation into the source of these PFAS. Analysis of raw industrial wastewaters in an upstream municipality revealed short-chain (C4-C7) PFCAs at levels insufficient to explain the extent of contamination. However, elevated concentrations of short-chain PFCAs were found in the corresponding wastewater treatment plant (WWTP) effluent relative to its influent, suggesting the presence of unknown PFAS precursors in raw wastewater. We employed the total oxidizable precursor (TOP) assay to assess PFAS precursor concentrations in textile wastewaters entering the WWTP. PFAS precursor concentrations near 10,000,000 ng/L were found in raw textile wastewaters after TOP assay, revealing the primary source of PFAS to the WWTP. A mass balance of WWTP influent and effluent revealed that over 90 percent of SCFP entering the WWTP was removed to sludge and eventual biosolids. TOP fingerprinting indicated that textile-associated precursors contained primarily 6:2 fluorotelomer-based PFAS functionality, but the application of high-resolution mass spectrometry (HR-MS) failed to yield the identity of these precursors. Ultrafiltration prior to TOP assay revealed that PFAS precursors in textile wastewater were colloidal in nature, as they passed through a 0.2 µm filter but were quantitatively retained by a 100 kDa ultrafiltration

membrane. Size separation and characterization of these isolated colloidal fractions using asymmetric field-flow fractionation (AF4) revealed them to be consistent with SCFP nanoparticles used in fabric finishing. In addition to wastewaters, SCFP from WWTP biosolids were extracted by sonication in a surfactant solution and were characterized by ultrafiltration and the TOP assay. The presence of colloidal PFAS in wastewater and their potential to degrade to molecular PFAS during transport poses significant challenges for receiving waters, downstream drinking water, and areas where biosolids are applied.

3.25.T-02 Evaluating Sorptive Removal of Novel Fluorochemicals from Lithium-Ion Batteries Using Commercial Sorbents

Faezeh Pazoki and Jinxia Liu, McGill University, Canada

The growing demand for lithium-ion batteries (LIBs), driven by electric vehicles and electronics, has raised concerns about their environmental impact, particularly the release of per- and polyfluoroalkyl substances (PFAS). These highly persistent and toxic compounds, used in LIB components such as electrolytes, binders, and separators, pose risks to water quality and ecological health. Although sorption technologies, including granular activated carbon (GAC), ion exchange resins (IXR), and clay-based sorbents like FluoroSorb-200, are commonly used for PFAS removal, their effectiveness for PFAS specific to LIBs has not been thoroughly evaluated. This study investigates the sorption behavior of seven lithium-based PFAS (LiBETI, LiTFSI, LiPF₆, LiBF₄, LiFSI, LiFTSI, and LiOTf) across deionized water, groundwater, and landfill leachates to assess sorbent performance under varying environmental conditions. Factors such as pH, ionic strength, and sorbent dosage were systematically studied to understand their influence on sorption efficiency.

Results demonstrated that all sorbents achieved high removal efficiencies (>95%) for larger PFAS, such as BETI, TFSI, FSI, and FTSI, in deionized water. For smaller PFAS, like BF₄, PF₆, and Triflate, IXR outperformed GAC and FluoroSorb, with BF₄ removal limited to ~60%. In complex matrices such as landfill leachates, GAC exhibited poor performance across all PFAS, with removal efficiencies dropping below 20% due to fouling by organic matter and competing ions. FluoroSorb maintained stable removal in groundwater but experienced a 10-30% reduction in landfill leachates. IXR demonstrated the highest overall efficiency, though Triflate sorption decreased by ~40% in groundwater and landfill leachates, likely due to competing anions.

Characterization techniques, such as XRD, FTIR and SEM, were employed to elucidate the sorption mechanisms. Isotherm and kinetic models were applied to quantify the sorption capacity and rate. Furthermore, the long-term stability of the sorbent-PFAS complexes was assessed under various conditions.

The findings of this study will contribute to a better understanding of the sorption behavior of emerging PFAS onto commercial sorbents in complex matrices.

3.25.T-03 To Treat or Not to Treat: Comparing Health Impacts of PFAS Exposure to Health Impacts of PFAS Removal Technologies

Sanne Julie Smith, Mar Palmeros Parada and Emile Sylvestre, Delft University of Technology (TU Delft), Netherlands

Associations between PFAS and adverse health effects have led to the global introduction of drinking water concentration limits in the low ng/L range. PFAS exposure has been shown to contribute considerably to disease burden, so interventions are clearly necessary to reduce exposure. However, to adequately quantify the health benefits of intensified drinking water treatment, the health effects of the treatment technologies should be considered as well. Therefore, the aim of this study was to estimate both types of human health impacts, i.e. the health gained by reduced PFAS exposure via drinking water and the health lost due to the drinking water treatment technologies, and quantify these in disability-adjusted life years (DALYs).

We performed a life cycle assessment to quantify the health lost (in DALYs) due to an increased regeneration frequency of granular activated carbon (GAC), which is used at a local drinking water producer to meet recent PFAS guidelines. To quantify the health gained by lower PFAS exposure, we first used the existing physiologically based pharmacokinetic model by EFSA to relate EFSA4 concentrations in drinking water to those in blood serum. Serum concentrations were then used in exposure response relationships from literature to relate them to an increase in disease occurrence, which was subsequently related to DALYs.

For all endpoints considered, we found that the gain in human health by removing PFAS from drinking water was in the same range as the loss of human health from the increased GAC regeneration. While the high uncertainty in PFAS health effects limits our ability to make a reliable comparison, it is likely that

other interventions that limit PFAS exposure have a higher net benefit than drinking water treatment. For example, phasing out all non-essential uses of PFAS will lead to a decreased exposure via multiple routes, including diet. Altogether, PFAS limits in drinking water may need to be determined on a case-by-case basis, that considers the current concentration levels in addition to the secondary impact of the required treatment technologies. This study mainly serves to start a dialogue about this complex issue, which is particularly important as increasingly many PFAS are added to drinking water guidelines, most of which are even more challenging to remove than those currently included.

3.25.T-04 Unveiling PFAS in Cosmetics: Global Insights and Australian Market Analysis Using Non-Target and Suspect Screening Analysis

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Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals valued for their unique physicochemical properties, making them integral to many consumer products. However, growing evidence of their toxicity, environmental persistence, and presence in cosmetics and personal care products (PCPs) has raised significant health and environmental concerns. Despite these risks, comprehensive data on PFAS prevalence in cosmetics remain sparse, leaving critical gaps in understanding consumer exposure.

The current study addresses these gaps through a twofold investigation. First, a scoping review assessed global studies of PFAS in cosmetics and PCPs. Second, the first-ever suspect screening and non-target analysis (NTA) using high-resolution mass spectrometry (HRMS) was conducted on cosmetic products available in Australian retail stores. The scoping review identified Polyfluoroalkyl phosphates (PAPs) as the most frequently detected PFAS, with an average concentration of 26.1 ppm, followed by perfluorooctane sulfonamides (FOSA, 4.02 ppm) and fluorotelomer sulfonamide surfactants (FTS/FTSA, 2.23 ppm). Sanitary pads, foundation products, and paper diapers were most commonly tested, while a single bronzer sample exhibited the highest concentration. Other products with notable PFAS levels included nightguards, whitening guards, and dental floss, highlighting diverse sources of exposure. The Australian analysis revealed that most of the tested products, including those without listed fluorinated ingredients, were positive for PFAS. PAPs were the most frequently detected compounds, consistent with global findings. Foundation products demonstrated the highest detection rates, followed by lipstick, hair oil, bronzer, and masks.

This research underscores the critical need to understand PFAS contamination in cosmetics and its potential contribution to human exposure. By linking specific PFAS compounds to widely used products, this study provides valuable insights for public health advocacy, regulatory strategies, and consumer education. Addressing these gaps is essential to mitigate exposure risks, guide safer product formulations, and inform future policies. This presentation combines novel data on Australian cosmetics with a global perspective, highlighting the urgent need for regulatory and consumer-focused action on PFAS in PCPs.

3.25.P-Tu280 Rapid Screening and Targeted Analysis of PFAS in Feminine Hygiene Products

Alyssa Wicks¹, Sydney K. Brady², Heather Whitehead¹, Thomas Hedman¹, Alison Zachritz³, Marta Venier² and Graham F. Peaslee¹, (1)University of Notre Dame, United States, (2)Indiana University, United States, (3)University of Notre Dame, United States

Per- and polyfluoroalkyl substances (PFAS) are a class of over 14,000 anthropogenic compounds that provide appealing physical properties such as water and oil repellency, and stain resistance. This in turn has led to the addition of PFAS to consumer products, including personal care products. While one study has investigated the use of PFAS in Chinese personal hygiene products, including feminine hygiene specific products, no such survey has been published for products sold in other markets. This work presents an investigation into the occurrence of PFAS in numerous types of feminine hygiene products, including sanitary pads, panty liners, incontinence underwear, menstrual cups, reusable pads, period underwear, and reusable incontinence underwear, purchased in North America and Europe.

First, particle-induced gamma-ray emission (PIGE) spectroscopy was used to screen all 78 feminine hygiene products for the presence of fluorine. PIGE is an ex-vacuo, nondestructive isotope analysis technique that screens products for their total fluorine concentrations as a surrogate for PFAS in just three minutes per sample. Then a subset of samples underwent targeted PFAS analysis of 31 analytes using liquid chromatography tandem mass spectrometry (LC-MS/MS) and an additional 12 analytes using gas chromatography-mass spectrometry (GC-MS).

Total fluorine screening identified intentional fluorination use (>110 ppm F) in at least one of the tested disposable pads, reusable pads, tampon applicators, disposable incontinence underwear, reusable incontinence underwear and plastic wrappers. Interestingly, all product categories contained at least one

product without intentional fluorine concentrations. For the subset of products that underwent targeted analysis, total targeted PFAS concentrations ranged from 1.4-4500 ng/g, with a median 188 ng/g. Neutral PFAS, especially FTOHs and FTMAcRs, contributed the greatest percentage to the sum of targeted PFAS. While PFAS were observed in some feminine hygiene products, it does not appear to be an essential component of any of these products. This is supported by the total F results returning at least one product in most categories that did not contain intentional fluorination. Additionally, minimal differences observed in total F and targeted profiles of North American vs European products suggests that PFAS use in these products is widespread.

3.25.P-Tu283 High-Resolution Mass Spectrometry Screening of Per- and Polyfluoroalkyl Substances (PFAS) in Plastic Products

Xiaoyuan Guo¹, Guomao Zheng¹ and Chenglin Liu², (1)Southern University of Science and Technology, China (Mainland), (2)YanTai University, China (Mainland)

Per- and polyfluoroalkyl substances (PFAS) have been widely used in commercial and industrial applications, including plastics, due to their outstanding properties of chemical resistance and surface activity. Although the role of PFAS as surfactants in numerous consumer products is well known, the presence of PFAS in plastic products as additives has been seldom reported. Given the ubiquity of plastics, the use of PFAS in plastics should be of concern to avoid unexpected PFAS release. In this study, 106 plastic products representing 11 types of different plastics were chosen to examine the presence of PFAS using suspect screening and nontarget analysis by high-resolution mass spectrometry. A total of 24 PFAS from 8 classes were identified at confidence levels of 2 or above, including novel PFAS such as hydrogen-substituted perfluoroalkane carboxylates (H-PFCA), N-methyl perfluoroalkane sulfonamidoacetic acids (MeFASAA) and bis-perfluoroalkyl sulfonimides (bis-FASIs). Perfluorobutane sulfonic acid (PFBS) was the most prevalent, detected in all types of plastics and displayed relatively high detection frequencies (100% and 94%, respectively) in samples of polyamide (PA) and polycarbonate (PC). The result of subsequent quantitative analysis exhibited that the concentrations of PFBS in PC ranged from 0.2-6130 ng/g. The relatively high PFBS concentrations were mostly observed in plastic products with fire-resistant properties, implying the role of PFBS in achieving specific functions. In addition, 3H-perfluorobutane sulfonic acid (H-PFBS), a barely reported hydrogen-substituted perfluoroalkane sulfonate, was identified in samples associated with high PFBS concentration, which is regarded as the byproduct of PFBS manufacturing. A plastic-aging simulation by natural solarization and water immersion was performed to evaluate the potential of PFBS leaching from plastic products. The average leaching rate of PFBS after a 4-day simulation was 33%, indicating the great potential of PFBS to be transferred from plastic products to the environment. Overall, this study provides a crucial reference of PFAS presence in plastic products and suggests that plastics might constitute a potential source of PFAS release. Therefore, the proper use of PFAS in plastic manufacturing and stringent regulation for the disposal of wasted plastics should be emphasized.

3.25.P-Tu286 Fate and Effects of Perfluoroalkyl Substances and their Alternatives in the Marine Environment

Aasim Ali¹, Monica Sanden¹, Stig Valdersnes¹, Stepan Boitsov¹, Bjorn Einar Grosvik¹, Liv Softeland¹, Roland Kallenborn², Leiv Kristen Sydnes³, Christopher Higgins⁴, Nachiket P. Marathe⁵, Camilla Kirkeli⁶, Sylvia Frantzen⁵, Are Saele Bruvold⁵, Bente Nilsen¹ and Julia Storesund¹, (1)IMR Norway, Norway, (2)NMBU-Norway, Norway, (3)UiB-Norway, Norway, (4)Civil and Environmental Engineering, Colorado School of Mines, United States, (5)Institute of Marine Research (IMR), Norway, (6)University of Bergen-Norway, Norway

Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic chemicals comprising thousands of related compounds. They are of significant environmental concern due to their persistence, potential for bioaccumulation, and toxicity. Consequently, the use of many PFAS compounds has been banned, leading to the emergence of fluorinated and non-fluorinated alternatives in various products, such as firefighting foams. However, the fate and effects of these alternatives remain poorly understood.

Our project, FEARLESS, aims to enhance our understanding of how marine organisms are impacted by PFAS and their alternatives. We focus on the transfer and transformation of PFAS and their precursors within marine food webs. FEARLESS emphasizes developing advanced analytical methods to quantify PFAS and their precursors and alternatives, assessing their toxicity and bioaccumulation in marine organisms under both laboratory and field conditions, and studying their distribution in waters influenced by firefighting foams and other point sources.

We have selected Norwegian waters influenced by runoff from onshore (Bergen Airport) and offshore

(Ekofisk and Eldfisk oil fields) point sources as our research platforms, with locations distant from known sources serving as reference sites.

This presentation will introduce the FEARLESS project and share preliminary results. Implications and consequences including the evaluation of regrettable replacement of previously banned hazardous PFASs will be discussed.

Disclaimer/Disclosure: Research council of Norway - Project Number: 335543 Fate and effects of perfluoroalkyl substances and their precursors and alternatives in Norwegian marine environments and seafood species (FEARLESS).

3.25.P Emerging and Novel Per- and Polyfluoroalkyl Substances (PFASs): Latest Findings and Innovation Towards Safe and Sustainable Alternatives

3.25.P-Tu278 A Green Transition? An Overview of Current Uses of Per- and Polyfluoroalkyl Substances in the Shift from Fossil Fuels to Green Energy

Amanda Rensmo¹, **Juliane Gluge**², **Carla A. Ng**³, **Martin Scheringer**⁴, **Zhanyun Wang**⁵, **Xenia Trier**⁶, **Rainer Lohmann**⁷, **Sharyle Patton**⁸ and **Ian Cousins**¹, (1)Department of Environmental Science, Stockholm University, Sweden, (2)Institute of Biogeochemistry and Pollutant Dynamics (IBP), Department of Environmental Systems Science, ETH Zurich, Switzerland, (3)Department of Civil and Environmental Engineering, University of Pittsburgh, United States, (4)Department of Environmental Systems Science, ETH Zurich, Switzerland, (5)EMPA - Swiss Federal Laboratories for Materials Science and Technology, Switzerland, (6)Department of Plant and Environmental Sciences, Section for Environmental Chemistry and Physics, University of Copenhagen, Denmark, (7)Graduate School of Oceanography, University of Rhode Island, United States, (8)Health and Environment Program Commonwealth, CA, United States

Extensive research on climate change over the years all points in the same direction; systemic action is increasingly urgent. As a response, substantial efforts have been launched for a transition from fossil fuels to renewable alternatives, which includes the electrification of the energy, transportation and industry sectors, among other solutions. Solar panels, electric vehicles and heat pumps are some examples in this so-called green energy transition.

Still, the notion of green is without a conclusive definition. To contextualize, pollution and biodiversity loss, apart from climate change, have been shown to threaten the environment, ecosystems and human wellbeing, jointly known as the triple planetary crisis. The three dimensions (and the solutions to them) are interlinked, as can be exemplified through the use of per- and polyfluoroalkyl substances (PFAS). While they may provide important functions in the proposed green energy solutions to mitigate climate change (e.g. as binder in batteries), all PFAS in this immense chemical class are persistent, leading to significant, long-lasting pollution impacts. Thus, the question remains, can we make the current renewable energy transition a truly green energy transition? To address this matter, it is key to first understand if PFAS are essential in a green energy transition.

In this work, we present an overview of the uses of PFAS in several major energy technologies that commonly are rated as green. This includes, for instance, various battery chemistries, energy generation applications such as photovoltaics and wind turbines as well as fuel cells and electrolyzers of different types. Furthermore, we point to the need for transition to alternatives. The data were collected with an extensive literature review of both peer-reviewed literature as well as regulatory texts, surveying industrial and academic contributions to the field. In addition, interviews were conducted with both current industry parties which use PFAS and new actors providing alternative solutions.

Lastly, we emphasize the importance of highlighting the broader impacts, including PFAS pollution, associated with implementing green energy technologies to address climate change. This work contributes to this understudied field by providing knowledge and data that can be used to inform policy - and decision-making, such as improving recycling to mitigate potential emissions of PFAS or advancing alternative assessments of these technologies.

3.25.P-Tu279 Improved Prediction of Partitioning Properties for Data-Poor PFAS (Per- and Polyfluoroalkyl Substances)

Trevor N. Brown, **Jon A. Arnot** and **Alessandro Sangion**, ARC Arnot Research and Consulting Inc. and University of Toronto, Canada

The accurate prediction of partitioning properties of per- and polyfluoroalkyl substances (PFAS) is a current research need because these properties are required for hazard and risk assessment worldwide. However, there are limited experimental partitioning data for PFAS available, and many novel PFAS are data-poor with no measured properties. Data-drive property prediction tools such as quantitative structure-

property relationships (QSPRs) have poor predictive power for novel PFAS because of this lack of experimental data. Recently, new empirically calibrated partitioning property values for PFAS have become available in the form of poly-parameter linear free energy relationships (PPLFERs). Our open source QSPR package IFSQSAR has been updated with new data and PPLFER equations for fundamental partitioning properties including the octanol-water, octanol-air, and air-water partition ratios. IFSQSAR predictions can be accessed in the free to use EAS-E Suite online platform. The mechanistic basis of PPLFERs allows for an in-depth analysis of the unique partitioning properties of PFAS and the volume term was found to be particularly important. The updated IFSQSAR improved performance vs. experimental and empirical data for PFAS properties where available. A dataset of data-poor PFAS with partitioning properties calculated with quantum chemistry software was also used as an additional external check of predictive power. IFSQSAR showed good agreement with this external check compared to other QSPR packages that do not yet incorporate the new partitioning property data. The inclusion of the new PPLFER data and the mechanistic insights from this work could be used to help improve the predictive power of other QSPRs, and to prioritize future experimental work.

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3.25.P-Tu280 Rapid Screening and Targeted Analysis of PFAS in Feminine Hygiene Products

Alyssa Wicks¹, Sydney K Brady², Heather Whitehead¹, Thomas Hedman¹, Alison Zachritz³, Marta Venier² and Graham F. Peaslee¹, (1)University of Notre Dame, United States, (2)Indiana University, United States, (3)University of Notre Dame, Notre Dame, United States

Per- and polyfluoroalkyl substances (PFAS) are a class of over 14,000 anthropogenic compounds that provide appealing physical properties such as water and oil repellency, and stain resistance. This in turn has led to the addition of PFAS to consumer products, including personal care products. While one study has investigated the use of PFAS in Chinese personal hygiene products, including feminine hygiene specific products, no such survey has been published for products sold in other markets. This work presents an investigation into the occurrence of PFAS in numerous types of feminine hygiene products, including sanitary pads, panty liners, incontinence underwear, menstrual cups, reusable pads, period underwear, and reusable incontinence underwear, purchased in North America and Europe.

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3.25.P-Tu281 Comprehensive Characterization of Side-Chain Fluorinated Polymers in Consumer Products via Pyrolysis-GC-HRMS and NMR

Racchana Ramamurthy¹, Min Liu¹, Sebastian Sauve² and Jinxia Liu³, (1)McGill University, Montreal, Canada, (2)University of Montreal, Canada, (3)McGill University, Canada

Per- and polyfluoroalkyl substances (PFAS) are widely utilized in consumer and industrial applications due to their unique physicochemical properties. While research and regulatory efforts have predominantly focused on low molecular weight, non-polymeric PFAS, polymeric PFAS comprising the majority of PFAS products remain underexplored. Side-chain fluorinated polymers (SCFPs) are particularly

concerning as they degrade into environmentally persistent perfluoroalkyl acids.

SCFPs have been identified in Scotchgard impregnation sprays, with studies reporting their presence in sediments and biosolid-amended soils at concentrations up to thirty times higher than those of non-polymeric PFAS. Detecting SCFPs in environmental samples is challenging due to low extractability, the lack of chemical standards, and ionization difficulties under electrospray ionization.

This study investigates the chemical composition and polymer chemistry of 11 commercially available Scotchgard products spanning 1987 to 2022, including fabric and carpet cleaners and protectors. Pyrolysis-GC-HRMS, a novel technique requiring minimal sample preparation, was employed to characterize the bulk polymer types by analyzing pyrolysis products generated at 700 °C in single-shot mode. Pyrolysis products confirmed the presence of side-chains containing perfluorobutane or perfluorooctane sulfonamides. LC-HRMS analysis of non-polymeric PFAS indicated high amounts of PFHxS, PFOS and PFBS. One-dimensional ¹⁹F NMR revealed a transition in formulations post-2000 from C8-ECF to C4-ECF-based PFAS. Two-dimensional DOSY NMR further supported these observations, showing diffusion coefficients intermediate between non-polymeric and polymeric PFAS. DOSY NMR performed after hydrolysis experiments demonstrated that SCFPs degrade into non-polymeric PFAS, as evidenced by changes in diffusion coefficients post-hydrolysis. We use the information to piece together the chemical compositions of PFAS in these products for the first time. These findings underscore the environmental persistence and transformation potential of SCFPs in consumer products, highlighting the urgent need for further research into their environmental behavior, risks, and long-term impacts.

3.25.P-Tu282 Routine PFAS Testing of Surface Water Samples Using TOP Assay and ACQUITY™ QDa™ II Mass Detector

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Per- and polyfluoroalkyl substances (PFAS) have increasingly become a major environmental and public health concern due to their toxic properties and tendency to bioaccumulate in living organisms, such as crops and wildlife. Global widespread use of PFAS over many decades has meant that these compounds have become common and persistent environmental pollutants. Contamination of rivers, groundwater, and drinking water sources with PFAS has been reported globally. Concern in the United Kingdom has been amplified recently by increasing reports of wastewater and sewage leaks into rivers and other water bodies; this discharge of untreated or partially treated wastewater into rivers can exacerbate trophic transfer and persistence throughout vital ecosystems and food chains. The resistance of PFAS to traditional environmental remediation techniques makes addressing their impacts particularly challenging. Traditional testing methods often fail to capture the full extent of PFAS pollution, primarily detecting legacy PFAS compounds and overlooking the myriads of precursor compounds and non-legacy PFAS compounds. The total oxidizable precursors (TOP) assay approach offers a means of bridging this gap; by oxidizing unknown PFAS precursors and intermediates and converting them into a limited set of well-characterized stable perfluoro-carboxylic and perfluoro-sulfonic acids, it allows for a more complete insight into complex environmental samples. By utilising an LC-MS approach with the ACQUITY QDa II Mass Detector, this method provides a cost-effective means of risk assessment for environmental samples. It complements traditional testing methodologies by enhancing the identification and assessment of both legacy PFAS compounds and perfluoroalkyl acid (PFAA) precursors in each sample.

In this work, eight surface water samples were collected downstream from wastewater treatment plants across the northwest of England. By comparing perfluoroalkyl carboxylic acids (PFCA) concentrations before and after oxidation, this method allows for the assessment of total PFAA precursor content in a given sample. All surface water samples tested showed a significant increase in total PFCA content post-amendment, with in-sample concentrations ranging from 67 to 2711 ng/L (ppt) - indicating a high degree of precursor contamination that traditional analytical methods may have overlooked.

3.25.P-Tu283 High-Resolution Mass Spectrometry Screening of Per- and Polyfluoroalkyl Substances (PFAS) in Plastic Products

Xiaoyuan Guo¹, Guomao Zheng¹ and Chenglin Liu², (1)Southern University of Science and Technology, China (Mainland), (2)YanTai University, China (Mainland)

Per- and polyfluoroalkyl substances (PFAS) have been widely used in commercial and industrial applications, including plastics, due to their outstanding properties of chemical resistance and surface

activity. Although the role of PFAS as surfactants in numerous consumer products is well known, the presence of PFAS in plastic products as additives has been seldom reported. Given the ubiquity of plastics, the use of PFAS in plastics should be of concern to avoid unexpected PFAS release. In this study, 106 plastic products representing 11 types of different plastics were chosen to examine the presence of PFAS using suspect screening and nontarget analysis by high-resolution mass spectrometry. A total of 24 PFAS from 8 classes were identified at confidence levels of 2 or above, including novel PFAS such as hydrogen-substituted perfluoroalkane carboxylates (H-PFCA), N-methyl perfluoroalkane sulfonamidoacetic acids (MeFASAA) and bis-perfluoroalkyl sulfonimides (bis-FASIs). Perfluorobutane sulfonic acid (PFBS) was the most prevalent, detected in all types of plastics and displayed relatively high detection frequencies (100% and 94%, respectively) in samples of polyamide (PA) and polycarbonate (PC). The result of subsequent quantitative analysis exhibited that the concentrations of PFBS in PC ranged from 0.2-6130 ng/g. The relatively high PFBS concentrations were mostly observed in plastic products with fire-resistant properties, implying the role of PFBS in achieving specific functions. In addition, 3H-perfluorobutane sulfonic acid (H-PFBS), a barely reported hydrogen-substituted perfluoroalkane sulfonate, was identified in samples associated with high PFBS concentration, which is regarded as the byproduct of PFBS manufacturing. A plastic-aging simulation by natural solarization and water immersion was performed to evaluate the potential of PFBS leaching from plastic products. The average leaching rate of PFBS after a 4-day simulation was 33%, indicating the great potential of PFBS to be transferred from plastic products to the environment. Overall, this study provides a crucial reference of PFAS presence in plastic products and suggests that plastics might constitute a potential source of PFAS release. Therefore, the proper use of PFAS in plastic manufacturing and stringent regulation for the disposal of wasted plastics should be emphasized.

3.25.P-Tu285 Simultaneous Detection of Legacy and Emerging PFAS: Gas/Particle Distribution and Partitioning in Seoul

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Poly- and perfluoroalkyl substances (PFASs) are persistent pollutants widely detected across various environmental matrices, raising global concern due to their persistence and potential health risks. This study aims to quantify the concentrations of PFASs in the atmosphere of Seoul and assess their gas/particle partitioning behavior, with a focus on providing insights critical to air quality management and regulatory strategy development. The primary challenge addressed is determining the concentration profiles and distribution patterns of different PFAS compounds in Seoul's atmosphere, focusing on their partitioning between gas and particle phases.

To achieve this, atmospheric samples were collected from February 10 to March 16, 2024, at the Korea Institute of Science and Technology (KIST) in Seoul, using high-volume air sampling equipment. Particulate-phase PFASs were captured on quartz fiber filters, while gas-phase compounds were collected using polyurethane foam (PUF)/activated carbon felt (ACF)/PUF sandwiches. Sample analysis was conducted via high-performance liquid chromatography coupled with a triple quadrupole mass spectrometer (HPLC-MS/MS), which enabled the quantification of individual PFAS compounds. Results indicate that short-chain PFASs, including perfluorobutanoic acid (PFBA) and perfluorobutanesulfonic acid (PFBS), were the most prevalent, with a tendency to exist in the gas phase, suggesting higher volatility and potential for long-range atmospheric transport. Conversely, long-chain PFASs like perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) were predominantly associated with particles, indicating a greater tendency to remain localized. The total concentration of PFASs ranged from 3.19 to 29.2 pg/m³, with a median of 5.42 pg/m³. These findings highlight distinct atmospheric behaviors based on PFAS chain length and contribute valuable data for urban air quality management. Ongoing monitoring is essential to fully assess the environmental impact of legacy and emerging PFASs.

3.25.P-Tu286 Fate and effects of perfluoroalkyl substances and their alternatives in the marine environment

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Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic chemicals comprising thousands of related compounds. They are of significant environmental concern due to their persistence, potential for bioaccumulation, and toxicity. Consequently, the use of many PFAS compounds has been banned, leading to the emergence of fluorinated and non-fluorinated alternatives in various products, such as firefighting foams. However, the fate and effects of these alternatives remain poorly understood.

Our project, FEARLESS, aims to enhance our understanding of how marine organisms are impacted by PFAS and their alternatives. We focus on the transfer and transformation of PFAS and their precursors within marine food webs. FEARLESS emphasizes developing advanced analytical methods to quantify PFAS and their precursors and alternatives, assessing their toxicity and bioaccumulation in marine organisms under both laboratory and field conditions, and studying their distribution in waters influenced by firefighting foams and other point sources.

We have selected Norwegian waters influenced by runoff from onshore (Bergen Airport) and offshore (Ekofisk and Eldfisk oil fields) point sources as our research platforms, with locations distant from known sources serving as reference sites.

This presentation will introduce the FEARLESS project and share preliminary results. Implications and consequences including the evaluation of regrettable replacement of previously banned hazardous PFASs will be discussed.

Disclaimer/Disclosure: Research council of Norway- Project Number: 335543 Fate and effects of perfluoroalkyl substances and their precursors and alternatives in Norwegian marine environments and seafood species (FEARLESS) .

3.25.P-Tu287 Per- and Polyfluoroalkyl Substances (PFAS) Levels in Urban Stormwater Runoff Demonstrate the Importance of Outdoor Sources

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On behalf of the Regional Monitoring Program (RMP) for Water Quality in San Francisco Bay (California, USA), we evaluated the occurrence of per- and polyfluoroalkyl substances (PFAS) in stormwater runoff. The RMP started monitoring PFAS in 2006, generating data that now rank the class as a high concern in San Francisco Bay due in part to fish tissue concentrations that exceed some human health risk thresholds. In urban to peri-urban watersheds such as those surrounding San Francisco Bay, stormwater runoff is a major pathway by which contaminants enter aquatic ecosystems. Like >85% of the USA population, the population in this region has separated outdoor (stormwater) and indoor (sewer) drainage systems. Time-averaged composite sampling of stormwater runoff focused on storms in highly developed watersheds, with complementary sampling in less-urban reference watersheds, for a total of more than 30 sample sets over five wet seasons. Samples were analyzed for the 40 PFAS listed in EPA Method 1633 via liquid chromatography coupled to tandem mass spectrometry, with subsets of samples subjected to more extensive targeted analysis of analytes such as polyfluoroalkyl phosphate diesters (diPAPs), and the total oxidizable precursors (TOP) assay to measure unknown precursors. In initial data, most frequent detections were of perfluorocarboxylic acids (PFCAs), perfluorosulfonic acids (PFSAs), and diPAPs. Measured concentrations rivaled those in effluents from the region's municipal wastewater treatment plants. In addition to firefighting foams and metal-plating mist suppressants, PFAS sources particularly relevant to outdoor urban environments include outdoor paints and coatings, roofing materials, textiles, solar panels, artificial turf, and automotive products. (The specific PFAS used in many of these products likely need to undergo transformation to form the intermediate and terminal products, like PFCAs and PFSAs, analyzed in this study.) Rainwater may contribute to PFAS in stormwater, though concentrations reported in rainwater elsewhere were in the lower range of concentrations detected in this study, and observed PFAS concentrations were generally significantly higher in highly developed watersheds relative to reference watersheds. PFAS sources, transformation, and transport processes will need more detailed studies to inform management of this complex pollutant class. Results emphasize the role of urban stormwater runoff in PFAS transport to surface waters.

3.25.P-Tu288 Emerging Patterns and Distributions of Select Per- and Polyfluoroalkyl Substances (PFAS) in Nebraska Drinking Water

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Per- and polyfluoroalkyl substances (PFAS) have dispersed widely into the environment from industrial and consumer uses, threatening water quality across the globe. Multiple research groups have detected PFAS in tap and bottled water in the United States (US) and many other countries. Pattern and concentrations of PFAS are influenced by water source, location, environment, and many other factors. At present there is limited information on PFAS occurrence in private wells. The primary objective of this research is to sample and measure PFAS in residential private and public water sources located around known or suspected point sources in Nebraska to characterize the risk of PFAS exposure through drinking water. Water samples were collected from randomly selected, mostly rural residences around seven different sites in Nebraska. Samples were extracted and analyzed by liquid chromatography tandem mass spectrometry following US Environmental Protection Agency (USEPA) Method 1633. Participants were surveyed at the time of water sample collection to help evaluate factors which may contribute to exposure. Well water PFAS concentrations and survey variables will be used to generate a USEPA hazard index (HI) to identify households and risk factors contributing to elevated risk. Preliminary results reveal that at least one PFAS compound was present above the detection limit in 89% of the water samples (N = 84). The most frequently detected PFAS were PFOSA (83%), PFOA (52%), PFBS (43%), PFHxS (42%), and PFBA (23%). The distribution of PFAS compounds differed by location, and regulated concentrations were below the USEPA maximum contaminant levels (MCLs). Eighty percent of the samples were collected from private wells. Fifty-five percent of participants reported their water was being treated. The most common types of water treatment were water softener (47%) and reverse osmosis (30%). Preliminary results provide quantified levels of PFAS in several residential water samples, with emerging patterns and distributions of PFAS in rural Nebraska. The next steps in this project include geospatial analysis, social vulnerability and health outcome analysis, and HI assessment for better prediction of drinking water exposure routes.

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3.25.P-Tu289 Wildlife Affected by PFAS from Remote Areas in Central Europe

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With the wide use and large number per and polyfluoroalkyl substances (PFAS), paired with their physical and chemical properties originating from the high C-F bond energy makes them persistent in the environment. Despite regulatory measures phasing out several PFAS, their presence in the environment is still detectable due to their long half-life, and transformation of precursor compounds. Wildlife in particular are vulnerable to PFAS exposure through the uptake of contaminated food and water, leading to the bioaccumulation of PFAS in various tissues. This makes wildlife valuable sentinel animals for biomonitoring PFAS exposure to humans.

In this study we sampled livers, kidneys, and brains of animals from various diet and environments (national park, mountainous regions and urban areas to assess their PFAS burden by analysing extractable organofluorine (EOF) and total fluorine (TF) analyses alongside targeted quantification of over 31 PFAS compounds. The target analysis included legacy PFAS, emerging PFAS, and ultrashort-chain PFAS (chain length <C3), enabling the detailed characterisation of specific PFAS as well as sum parameters. This multi platform approach provides new insights into the PFAS exposure across a diverse ecological context. The results show that diet, but most importantly habitat influences PFAS pattern and concentrations observed in the tissues, highlighting the interaction between environmental exposure pathways and uptake. The results also highlights the importance of biomonitoring PFAS.

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3.25.P-Tu290 Tissue-Specific Biodistribution of Emerging Per- and Polyfluoroalkyl Substances (PFAS) for Biomagnification Estimation in a Process-Based Aquatic Trophic Model

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This study investigates the tissue-specific distribution of emerging per- and poly-fluoroalkyl substances (PFAS), including GenX derivatives and fluorotelomers, in fish subjected to chronic environmental exposure in the tropical coastal waters surrounding Singapore. Understanding the biodistribution of these compounds is essential for assessing their bioaccumulative behaviours and potential toxicological impacts. While extensive research has been conducted on legacy perfluoroalkyl acids and has identified the liver as the primary accumulation site, tissue-specific distribution of the emerging GenX and fluorotelomers remains poorly understood. These compounds differ from legacy PFAS due to their higher molecular weight and tendency to undergo biotransformation. This study quantifies the concentrations of five GenX compounds and six fluorotelomers in various fish tissues, including liver, muscle, intestine, bone, skin, and tail, using targeted LC-MS/MS analysis. The tissue-specific distribution factors and body burdens are individually assessed and incorporated into a site-specific process-based aquatic trophodynamic model to evaluate the role of individual tissues in the overall bioaccumulation process. By comparing model outputs with empirical data, this study refines biomonitoring strategies by identifying tissues most suitable for estimating trophic transfer and ecological burdens of GenX and fluorotelomers in aquatic ecosystems.

3.25.P-Tu291 Preliminary Assessment of the Zebra Mussel (*Dreissena polymorpha*) as a Sentinel Organism to Monitor Legacy and Emerging PFAS in an Urban River: A case Study on the Seine River (France)

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Per- and polyfluoroalkyl substances (PFAS) are ubiquitous in aquatic environments. A recent shift toward emerging PFAS is calling for new data on their occurrence and fate. Understanding their bioaccumulation and its drivers is fundamental for risk assessment purposes. Sentinel organisms, such as zebra mussels, may be used to provide an integrated measure of pollutant bioavailability over time and thereby monitor water quality.

Here, we present preliminary data on the use of the zebra mussel as a sentinel organism to monitor legacy and emerging PFAS in a major urban river, the Seine River (France).

Individuals collected from a reference lake were transplanted for three weeks at five locations along the Seine River and some of its tributaries following an expected upstream-downstream gradient. Three campaigns were performed in autumn (2020, 2021 and 2023). Campaigns took place in autumn (2020, 2021, and 2023). At the downstream sites, sediment traps were deployed to collect suspended particulate matter (SPM), and bed sediments were also sampled. A total of 36 PFAS were monitored, including legacy compounds such as PFCAs and PFSA's but also more emerging chemicals rarely monitored in biota such as FTSA's, ethers or sulfonamidoalkyl betaines.

Among the 36 analysed PFAS, 17 were detected in sediments and SPM. PFOS, PFDoDA, and 6:2 FTAB were systematically found. 5:3 FTCA was detected in 4/5 sites, while 8:2 FTAB appeared sporadically at two downstream sites. Alternative PFAS like 6:2 and 8:2 Cl-PFESA or GenX were absent, while ADONA was found at two sites at very low concentrations. Molecular patterns were dominated by 6:2 FTAB, followed by 5:3 FTCA and L-PFOS. This highlights a notable contribution from emerging PFAS. In exposed zebra mussels, PFAS levels were significantly higher than in controls (i.e. up to 70 ng/g wet weight). Contrary to previous results obtained on fish from this river stretch, the molecular profile of PFAS in mussel tissues was generally very similar to that observed in abiotic samples. In particular, there was a notable contribution from relatively reactive PFAS, i.e. 10:2 FTSA or 6:2 FTAB, probably due to the limited capacity of zebra mussels to metabolise xenobiotics. This pattern seems therefore a fairly accurate reflection of the exposure environment, making zebra mussels reliable indicators of environmental exposure. Additional data are being collected to strengthen the use of zebra mussels in PFAS biomonitoring.

3.25.P-Tu292 Toxicological Effects of PFOS and PFBS on Rainbow Trout: In Vitro and In Vivo Approaches

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Per- and polyfluoroalkyl substances (PFAS), such as perfluorooctanesulfonic acid (PFOS) and perfluorobutanesulfonic acid (PFBS), are environmental contaminants of concern due to their persistence and potential toxic effects on aquatic life. This study aims to evaluate the effects of PFOS and PFBS on rainbow trout (*Oncorhynchus mykiss*) using in vitro (liver and gut cells) and in vivo (whole organism) experimental models. The objectives were to compare the biological impacts of these substances and assess the relevance of in vitro systems for predicting whole-organism responses. Rainbow trout liver and gut cells were exposed to two concentrations of PFOS and PFBS in vitro using a co-culture experimental setup. This was conducted using a co-culture experimental design, wherein liver cells were cultured in wells of 6-well plates, while gut cells were maintained in mesh inserts within the same wells. Additionally, experimental setups included liver cells cultured with an empty insert (i.e., no gut cells) and a setup with liver cells only, to facilitate comparative analysis. In parallel, juvenile rainbow trout were exposed to these compounds through their diet under controlled laboratory conditions. RNA was extracted from both in vitro liver and gut cells and from liver and gut tissues in vivo. Biomarker analysis focused on gene expression changes related to oxidative stress, lipid metabolism, thyroid hormone regulation, inflammation, and detoxification pathways. The research advances our understanding of PFOS and PFBS toxicity in aquatic organisms and highlights the complementary roles of in vitro and in vivo approaches in ecotoxicological studies.

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3.25.P-Tu293 Deriving Soil Threshold Values for PFASs – Navigating Toxicity, Exposure, and Practical Limits

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Per- and polyfluoroalkyl substances (PFASs) pose a major challenge for chemical soil protection. Due to their decades-long use in various applications and their persistence, PFASs are ubiquitous in the environment and in humans. Additionally, effects on human health and ecosystems have been observed at concentrations commonly found in foodstuff or the environment.

Various countries are now developing soil threshold values for PFASs, using different frameworks focusing on ecotoxicity, human health, or background contamination levels. In Switzerland, a new framework has recently been developed and proposed to derive soil threshold values for agricultural soils. This framework has been applied to PFASs and several challenges have been identified.

Firstly, the selection of substances to prioritize is complex due to the diversity of PFASs. There are several thousand PFASs with different behaviour, occurrence, and toxicity. Second, the lack of data complicates the derivation at several points. For many PFASs, there are knowledge gaps, which include unknown levels for background contamination in soil or foodstuff, insufficient knowledge on transfer to agricultural crops or animals, or not yet established tolerable weekly intake (TWI). Thirdly, aligning soil threshold values with already existing guideline values, such as maximum levels in foodstuff, drinking water guidelines, or TWI is an additional challenge. Finally, the practical application of soil threshold values for PFASs is complicated by the widespread presence of PFASs in the environment. High background levels make it difficult to establish thresholds that protect human and environmental health without rendering many agricultural areas non-compliant and therefore no longer usable for food production. This illustrates the difficulty of reconciling protective standards with feasibility and enforceability.

This study addresses the above challenges and identifies ways to overcome them to establish feasible, science-based soil threshold values for PFASs. It additionally identifies research gaps critical to regulation, demonstrating how targeted research can support the development of improved soil threshold values.

3.25.P-Tu294 Enhancing Removal Efficiency of Short-Chained PFAS Using a Two-Step Treatment Train: Flocculation and Sorption

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The presence of ultra-short and short-chain per- and polyfluoroalkyl substances (PFAS) present significant challenges in treating complex landfill leachates. Organic matter in soil and landfill leachate competes

with PFAS for sorption sites on carbonaceous sorbents, leading to reduced treatment efficiency and increased treatment costs. While activated carbon is commonly used, biochar may provide a sustainable alternative due to its lower environmental footprint and reduced cost as it is produced from waste materials. This study evaluates whether a two-step treatment train combining coagulation and flocculation with sorption can enhance the removal of ultra-short and short-chain PFAS from contaminated landfill leachate. The coagulants and flocculants have been shown to precipitate most of the long-chain PFAS, while shorter chained PFAS are less efficiently removed. We hypothesize that removal of negatively charged short-chain PFAS will be achieved by sorption of complexes, formed by flocculants and short-chain PFAS remaining in suspension, to the mesoporous biochar. We further hypothesize that this two-step approach, i.e., flocculation followed by sorption, will be a cost-efficient and more environmentally friendly approach to addressing the challenge of short-chain PFAS in complex landfill leachate. Preliminary results show that 34-97% of targeted PFAS can be removed from landfill leachate via flocculation, with longer-chained PFAS being more efficiently removed than shorter ones. Follow up work is now investigating spiked samples with a coagulation and flocculation step followed by sorption using biochar. Key water parameters will be monitored throughout the process, with PFAS analyses conducted post-jar test and post-batch experiment.

We will examine the interaction of ultra-short, short- and long-chained PFAS with organic matter during treatment. The test will be optimized with spiked samples before testing with landfill leachate. Quality assurance and control measures include running parallels, method blanks, sample blanks, and other controls.

The effect of various factors on removal efficiency, including coagulant and flocculant materials and dosages, organic matter content, and PFAS profiles, will be presented. We anticipate this research will advance water treatment strategies for short-chained PFAS and provide deeper insights into their removal mechanisms when using coagulants and flocculants in combination with sorption using biochar.

3.25.P-Tu295 Soil Column Chromatography Coupled with Mass Spectrometry to Investigate the Sorption of Per- and Polyfluoroalkyl Substances (PFASs)

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The transport of per- and polyfluoroalkyl substances (PFASs) in soil environment has attracted public concern because of their adverse effects. This study introduced a soil column chromatography (SCC) approach combined with liquid chromatography mass spectrometry (LC-MS) to characterize the soil sorption behavior of PFASs. Although SCC is an established approach, the PFAS of concern need MS detection because they are not measurable with a UV detector. This method is expected to overcome existing challenges and facilitate the acquisition of comprehensive data sets. To do so, the specific aims of the study are 1) to validate the SCC method for non-fluorinated neutral and anionic chemicals by HPLC-UV and PDA analysis; 2) to apply the SCC for LC-MS analysis and compare the LC-MS data to HPLC-PDA data and 3) to obtain the sorption coefficient (K_{oc} , kg/L) for PFASs by using the measured retention times of PFASs. The results showed that a newly customized SCC device using a stainless-steel column with dimensions of 20 mm in length x 3 mm in internal diameter and silica (SiO₂, 3 μ m particle size) as a dilution material is optimal. A comparison of sorption data for the non-fluorinated neutral chemicals reveals that there is no significant difference between the HPLC-PDA and LC-MS systems, and those sorption data are consistent with the data reported in the literature. The obtained LC-MS data showed that no notable variation was observed between retention time of the test PFASs and the dead time of bromine as chosen tracer, indicating the weak sorption of the PFASs to the selected peat. This research shows the potential of coupling the SCC approach with MS to obtain soil sorption data for PFASs, which should contribute to risk assessment and chemical management practices.

3.25.P-Tu296 Liquid Chromatography Retention Factors as a Metric for Hydrophobic Sorption Properties of Anionic PFAS

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Partitioning and adsorption from water to non-aqueous phases are important processes that determine the environmental behavior and biological effects of contaminants. For neutral compounds, it is common practice to use the octanol/water partition coefficient (K_{ow}) as an indicator of solute hydrophobicity and predict the corresponding partition coefficients. However, many per- and polyfluoroalkyl substances (PFAS) exist as anions in water at environmental pH, rendering K_{ow} irrelevant as a hydrophobicity metric. In this study, the retention times of 39 anionic PFAS and 20 non-fluorinated anionic surfactants were measured in reversed-phase liquid chromatography using an octadecyl (C18)-bonded silica column

and evaluated as a potential hydrophobicity indicator.

Anionic PFAS and analogous non-fluorinated surfactants were dissolved in methanol and injected (1 µL) into a single quadrupole LC/MS equipped with a Kinetex EVO C18 column (5 µm, 2.1 mm i.d. × 30 mm) to measure retention times (tr). Isocratic elution was applied with methanol/water mixtures containing 10 mM ammonium acetate and 0.2 mM ammonia. The column dead time t₀ was measured using glyceric acid as a tracer. The retention factor k was calculated as (tr - t₀)/t₀. The value of k at 0% methanol (k₀) was extrapolated from k_s measured at higher methanol fractions (up to 80%). For long-chain PFAS, k₀ had to be extrapolated over long distances from measurements at high methanol concentrations. Nevertheless, by measuring a series of PFAS with different chain lengths and using a highly consistent log k increment by adding -CF₂- unit to the molecule, it was possible to obtain consistent k₀ values even for long-chain PFAS. The k₀ values obtained were compared with the soil organic carbon/water, phospholipid membrane/water, and air/water interfacial partition coefficients, showing a high logarithmic correlation in all cases. Since the measurement of k₀ is reproducible and accurate, it can be used to estimate various hydrophobicity-related partition coefficients of anionic PFAS.

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3.25.P-Tu297 Evaluating PFAS Adsorption with Potash Alum and Chitin-Modified Biochar

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants that threaten water quality and ecosystem health. This study investigates the adsorption efficiency of potash alum-modified biochar and chitin-modified biochar for removing PFAS from contaminated water. Potash alum was innovatively generated from recycled soda cans, demonstrating a sustainable approach to upcycling waste materials. Preliminary assessments suggest that the alum-modified biochar exhibits a more crystalline structure, potentially enhancing adsorption efficiency. The goal of this project is to target short-chain PFAS removal, as these compounds are more mobile and pose unique challenges for conventional remediation techniques. The hypothesis driving this research is that introducing Al³⁺ onto the biochar surface may shift its binding mechanism from primarily hydrophobic interactions to enhanced electrostatic attraction, improving adsorption of short-chain PFAS. Similarly, chitin-modified biochar, with its inherent amine and hydroxyl functional groups, is hypothesized to strengthen adsorption via hydrogen bonding and electrostatic interactions with PFAS functional groups. Surface characterization is ongoing to evaluate the effects of potash alum and chitin treatments on biochar properties, including surface area, porosity, crystallinity, and functional groups. Adsorption experiments will assess the performance of modified and unmodified biochar using a mixture of 10 PFAS compounds, comprising both short-chain carboxylic acids (PFBA, PFPeA, PFHxA, PFHpA) and long-chain carboxylic acids and sulfonates (PFOA, PFNA, PFDA, L-PFBS, PFHxS, PFOS). Percent removal of PFAS will be measured to determine the effectiveness of the modifications in enhancing adsorption performance. This research highlights the dual environmental benefits of PFAS removal and waste recycling through innovative biochar modification strategies. By focusing on short-chain PFAS, which are typically more difficult to adsorb, this work aims to contribute to eco-friendly and sustainable solutions for PFAS remediation. Findings from adsorption studies and surface characterizations will be presented at the conference, showcasing the potential of alum- and chitin-modified biochar as promising tools for addressing PFAS contamination in water systems.

3.25.P-Tu298 Contributions on the Development, Implementation and Assessment of SSbD Alternatives to PFASs in the Packaging Industrial Sector

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Comprising more than 4,700 chemicals, per and polyfluorinated alkyl substances (PFAS) are a group of man-made chemicals, widely used to provide water and oil repellency, that accumulate over time in humans and in the environment. Also known as forever chemicals, they are extraordinarily persistent in the environment and in our bodies, being toxic at extremely low levels (i.e. parts per quadrillion), posing significant risks to our health.

The EU's Chemicals Strategy for Sustainability puts PFAS policy in the spotlight. Nowadays, a number of PFAS are on the REACH Candidate List of substances of very high concern (SVHC), while several additional PFAS are on the list for evaluation. At the same time, the European Commission is committed to phasing out all PFAS, allowing their use only where they are proven to be irreplaceable and essential to society.

Regarding their use in surface treatments and coatings, PFAS are widely used to provide water and oil repellency for various applications as non-sticking utensil pan surfaces, water repellent and anti-soiling treatment for textile, food-contact anti-soiling coating on paper or cardboard packaging. Alternative PFAS-free products are currently available on the market, but not answering all market requirements in terms of long-term repellency properties (usually hydrophobic but not oleophobic) limiting their widespread use.

The BIO-SUSHY project aims at developing innovative coating solutions that will be designed to meet the policy ambition of the EU's chemical strategy for sustainability toward a toxic free environment. Under the scope of the project, bio-based thermoplastic powder coating formulations for the packaging industrial sector will be developed, following the Safe and Sustainable by Design (SSbD) methodology to ensure safety and environmental performance.

In this regard, an operating SSbD framework for strategic development of hazard-safe PFAS-free repellent bio-based hybrid coatings is being developed. BIO-SUSHY will contribute to the definition of the criteria in terms of safety and sustainability needed for the development of strategies applied to novel coatings. The consideration of safety and sustainability dimensions, the definition of their criteria applied to novel coatings, and the construction and demonstration of the SSbD Framework in which they are integrated, ultimately constitute guidance for the development of safer and more sustainable materials.

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3.25.P-Tu299 Implementing the Safe and Sustainable by Design (SSbD) Criteria at an Early Innovation Stage to Develop Biobased PFAS Alternatives for Textiles

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As a result of the ongoing regulation of per- and polyfluoroalkyl substances (PFAS), the textile industry is phasing out PFAS-based materials in its products. The lack of sufficient information about the human and environmental risks of alternatives has led to an uninformed substitution process. A key functional chemical, durable water repellents (DWRs) based on side-chain-fluorinated polymers, has often been replaced with silicone polymer DWRs. These are associated with the release of cyclic siloxanes, such as octamethylcyclotetrasiloxane (D4), which is classified as a substance of very high concern (SVHC). In other cases, alternatives marketed as PFAS-free or C0 chemistries have been used without providing information on their chemical hazards. The industry urgently needs better information about the hazards and environmental impacts to choose PFAS alternatives in textiles.

In the European project BIOSUSTEX, we are developing new biobased DWRs for cellulosic fabrics and aim to implement the Safe and Sustainable by Design (SSbD) framework suggested by the EU at an early innovation stage to guide these developments. At this low technology readiness level (TRL), a lack of information on toxicity, exposure risks, and inventory data for life cycle assessment (LCA) makes a sufficient SSbD assessment challenging so far. We present a preliminary methodology overview of how to incorporate new models based on in silico predictions and qualitative life cycle-based risk mapping approaches to assess the SSbD principles for PFAS alternatives in textiles during the design stage.

3.26.P Mercury in the Environment - Science to End Mercury Pollution

3.26.P-Tu300 Mercury in Seafood - A Persuasive Argument to Reduce Mercury Pollution from Small-scale Gold Mining Activities?

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Seafood products are an important source of protein and are rich in essential minerals, vitamins, and polyunsaturated fatty acids such as omega-3. However, despite the numerous health benefits gained from seafood consumption, these products can contain harmful metals, including mercury. Small-scale gold mining commenced on Buru Island, Maluku Province, Eastern Indonesia in 2011 and has resulted in

mercury contamination through the estuary and marine environment. Concern was raised by the Buru Island community and at nearby Ambon City about the mercury levels in their locally sourced seafood. We collected triplicate seafood samples from fish markets on Buru Island (12 species) and Ambon (15 species) and compared these with a reference data set from a fish market in Eastern Australia (19 species) and investigated total and methyl mercury concentrations in tissue and skin. Various Maximum Levels (MLs) permissible in seafood were exceeded in tissue and skin of individuals of several species from all market locations and were most strongly linked to trophic order, with the highest concentration recorded in muscle of the blacktip shark (4590 µg/Kg). Individuals with unusually elevated mercury had a high proportion in the form of methylmercury (44-99%). There was considerable variation between individuals of the same species. Consumption scenarios show how avoidance of some species can ensure diets remain below the provisional tolerable weekly intake (PTWI). Local health advisors promote the importance of dietary protein and in Maluku Province the marine environment is its major source.

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3.26.P-Tu301 Mercury on the Rise in Aquatic Biota from Freshwater and Marine Ecosystems: A South African Perspective

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Mercury (Hg) contamination in various aquatic ecosystems is a major concern as global emissions are increasing. These Hg emissions enter aquatic ecosystems via various anthropogenic sources, including artisanal and small-scale gold mining, coal combustion power stations and non-ferrous metal production. All of these major sources of Hg are occurring in South Africa (SA), resulting in SA being one of the top ten contributors of Hg emissions globally. When Hg enters the environment, it does not only act as a local contaminant but can be transported by long-distance atmospheric deposition, where our data have shown that it is readily accumulated in biota and biomagnified in food webs. This study determined the current Hg contamination status of SA in freshwater and marine ecosystems by assessing various environmental matrices and the transfer of Hg in the environment. It is evident that Hg concentrations in SA have increased over the last decade, in both the freshwater and marine environment, and is present in biota from these systems. When applying the USEPA human health risk assessment to consumption of fish, it was found that Hg can pose non-carcinogenic health risks to human consumers. Even though SA is a party to the Minamata Convention, that aims to reduce the effects of Hg on environmental and human health, more than 80% of the power generation in SA still relies on coal combustion power stations. Increased artisanal and small-scale gold mining activities also contribute to elevated Hg concentrations, especially in aquatic ecosystems. This study highlights the need to continuously monitor Hg concentrations in both freshwater and marine ecosystems to assess the exposure and effects of Hg on environmental and human health.

3.26.P-Tu302 Innovative High-Throughput Method for Ultratrace Methylmercury Detection in Biological Samples: A Breakthrough in Biomonitoring for Public Health Risk Assessment

Davide Spanu, Damiano Monticelli and Sandro Recchia, University of Insubria, Italy

Methylmercury (MeHg), a highly toxic neurotoxin, accumulates through the food chain, particularly via fish consumption, posing significant health risks. Current biomonitoring often measures total mercury (Hg), neglecting the selective detection of MeHg, which is critical for accurate exposure and risk assessment. This gap limits our understanding of MeHg's true impact on human health.

To address this challenge, we developed an innovative, rapid, and highly sensitive method for MeHg determination in fish tissue and human hair using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) paired with a custom low-pressure column. Unlike traditional methods requiring High-Performance Liquid Chromatography (HPLC), this streamlined approach employs an anion exchange resin that blocks inorganic Hg species, allowing for the selective elution of MeHg. This reduces complexity while maintaining high accuracy and efficiency.

Our method features a 15-minute ultrasound-assisted extraction with an optimized HCl-HBr-thiourea mixture, delivering a low detection limit of 0.05 µg/kg (in solid samples when using typical sample size of 100 mg) and minimizing matrix interference. Validation with certified reference materials, including fish muscle, liver, phytoplankton, and human hair, confirms its robustness across diverse biological samples. Application to marine trophic chains in Djibouti demonstrated a high trophic magnification factor (TMF = 13.5) for MeHg, emphasizing the severe risk posed by its biomagnification. Additionally, hair samples

from local populations showed a clear correlation between MeHg exposure and seafood consumption patterns, underscoring the method's potential for precise dietary exposure monitoring. This newly developed technique provides a powerful tool for high-throughput, selective MeHg analysis, supporting comprehensive biomonitoring programs aimed at mitigating public health risks associated with MeHg exposure.

3.26.P-Tu303 A [203Hg]-Metacinnabar Radiotracer to Model the Behaviour and Impacts of Mercury in Decommissioned Subsea Oil and Gas Pipelines

Elisabeth Tondl, Alexandra Boyd, Tom Cresswell and Francesca Gissi, ANSTO, Australia

Globally, a large amount of offshore oil and gas infrastructure is reaching end of production life. Subject to local legislative framework, once decommissioned the infrastructure may be left in the ocean or fully removed. Environmental contaminants such as mercury have been found in subsea pipelines at concentrations of up to five orders of magnitude above default sediment quality guideline values. Decisions on leaving infrastructure in situ should consider the behaviour and impact of mercuric species if they are released into the marine ecosystem as the pipes corrode.

Metacinnabar (γ -HgS, mercuric sulfide) is a nanoparticulate and poorly soluble form of mercury that has been found in subsea pipelines. Changes to the chemical speciation of the mercury mediated by organisms and sediment redox profiles may promote the formation of methylmercury, which is a neurotoxin that poses serious toxicological risks. The interaction of γ -HgS with seawater, sediments, and biota requires further investigation to ascertain risks posed by mercury if the pipelines are left in situ, then corrode and interact with the ecosystem.

Radiotracing is a technique that enables chemicals to be rapidly tracked and their concentrations to be quantified in model ecosystems under laboratory conditions to elucidate the behaviours of the contaminants. The mercury-203 (^{203}Hg) nuclide is radioactive and can be used to synthesise γ -[^{203}Hg]HgS for use as a model of γ -HgS in subsea pipelines. Literature methods for synthesis of radioactive γ -HgS have different formulations of the radioactive mercury reagent, and lack synthetic repeatability.

This presentation describes the development of a repeatable synthetic method for γ -[^{203}Hg]HgS, and presents results of seawater solubility experiments using the radiotracer γ -[^{203}Hg]HgS to inform assessment of the risks posed by leaving subsea pipelines that contain γ -HgS in situ.

3.26.P-Tu304 A Perspective on Mercury Methylation Promoted by Particulate Mercury Species in Aquatic Environments

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Mercury (Hg) is a global health concern due to the long-range intercontinental transport of elemental Hg in the atmosphere and microbial methylation of inorganic Hg to produce highly toxic and bioaccumulative methylmercury (MeHg) in aquatic environments. Mercury methylation is primarily governed by the activities of microbial communities and the availability of inorganic Hg for use by the methylators. Aquatic Hg can be present as dissolved Hg species and more prevalently as particulate Hg forms, including Hg-bearing minerals (e.g., metacinnabar, HgS) and Hg adsorbed on the surface of particles (e.g., iron minerals-bound Hg). Earlier studies have been focused on the bioavailability of dissolved Hg species for Hg-methylating microbes, but a series of our recent studies and others work have increasingly highlighted the role of particulate Hg species in promoting Hg methylation. By reviewing these recent studies, we hereby aim to provide a perspective on the new information regarding the Hg methylation processes involving particulate Hg species. First, some particulate Hg, e.g., HgS nanoparticles, could be directly available for bacterial uptake, serving as Hg substrate entering the cells and thus enhancing Hg methylation. Second, particle surface-adsorbed Hg could be taken up by Hg methylating bacteria co-adsorbed in the proximity through organic ligands released by the bacteria. Third, the close proximity of Hg species and Hg methylating bacteria on the particle surface could produce localized microenvironment with high microbial activity and readily available Hg, hence facilitating Hg methylation.

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3.26.P-Tu305 Mercury in a Low Trophic Level Prey-Predator System: The Freshwater Gammarid *Gammarus pulex* and Juvenile Brown Trout *Salmo trutta*

Poul Bjerregaard, Department of Biology, University of Southern Denmark, Denmark

Kinetics for inorganic mercury and methyl mercury were investigated in the freshwater gammarid *Gammarus pulex*. Upon exposure to radioactively labelled inorganic mercury, a steady state concentration factor of approximately 900 was reached in four days. For methyl mercury a steady state concentration factor of approximately 4000 was reached in 7 days. Retention of inorganic mercury and methyl mercury taken up from water over 8 days could be described as bi-exponential decay, with no statistically significant difference between the two mercury forms; 15-25% of the body burden were eliminated within two weeks.

Gammarids fed with food enriched with labelled methyl mercury for 8 days did not eliminate any of the mercury over 10 days; this was also true for gammarids exposed to selenite in both food and water during the elimination period.

G. pulex is an important food item for brown trout.

Juvenile brown trout were collected from 13 different streams on the Island of Funen, Denmark; none of the streams have any known history of mercury contamination. Muscle samples for mercury analysis were dissected out below the dorsal fin of the juvenile trout. The samples were freeze dried and concentrations of total mercury were determined by means of a Milestone Direct Mercury Analyzer.

Average mercury concentrations in the brown trout from the 13 streams varied from 148 to 348 ng Hg/g dry weight with highly significant differences between some of the individual streams. Considering that none of the streams have any known history of mercury contamination this came as a surprise. In one stream, mercury concentrations were determined in 56 fish, and mercury concentrations correlated ($r = 0.53$; $p < 0.001$) with the size (and probably age) of the fish.

From each of the two streams with the highest and lowest mercury concentrations, respectively, in the brown trout, fifteen *G. pulex* were collected for analysis of total mercury. The pattern for the brown trout was clearly reflected in the gammarids with average concentrations of 32 and 33 ng Hg/g in the low mercury levels streams and 63 and 76 ng Hg/g in the high level streams.

Mercury is biomagnified from gammarids to brown trout, and the results underline the necessity of elucidating the relative roles of methyl mercury and inorganic mercury in this process. This question is presently under investigation in our laboratory.

3.26.P-Tu306 Mercury Accumulation in Corbicula Clams: Insights into Freshwater Pollution in South Africa

Chelsea Withfield, Wynand Malherbe and Johannes Hendrik Erasmus, Water Research Group, UESM, North-West University, South Africa

South Africa is a significant global contributor to mercury (Hg) emissions, a critical concern due to its toxicity and severe health impacts on biota. Mercury enters freshwater systems primarily through anthropogenic activities, such as emissions from coal-fired power stations and artisanal gold mining. Bivalves are known to accumulate metals, including Hg, from their aquatic environments, making them potential bioindicators. The freshwater bivalve genus *Corbicula* is widespread and abundant in South Africa, offering potential for monitoring Hg pollution in freshwater ecosystems. This study sampled 35 sites across northeastern South Africa, detecting *Corbicula* clams at 15 locations. At each site, a minimum of five clams were collected along with water and sediment samples. Total Hg concentrations in these samples were analysed using a Flow Injection Mercury System. Mercury concentrations in sediment and water correlated with land-use activities, with sites closer to Hg sources showing elevated levels. Notably, environmental Hg concentrations in the Olifants and Inkomati Water Management Areas (WMAs) were higher than those reported 12 years ago. Mercury concentrations in *Corbicula* tissues exceeded those in corresponding sediment samples, with larger clams exhibiting higher Hg levels, possibly reflecting long-term exposure. A weak negative correlation between water and *Corbicula* Hg concentrations suggested that bioaccumulation is influenced more by sediment than water. This study underscores the potential of *Corbicula* clams as effective bioindicators, as Hg accumulation in their tissues offers a more comprehensive representation of bioavailable Hg in aquatic systems than sediment or water samples alone.

3.26.P-Tu307 Mercury in Offshore Oil and Gas Infrastructure; Informing Ecological Risk Assessments for Decommissioning

Tom Cresswell¹, Alexandra Boyd¹, Elisabeth Tondl¹, Marie Thomas² and Francesca Gissi¹, (1)ANSTO, Australia, (2)AIMS and The University of Queensland, Australia

Over recent years, there has been an increased global focus on strategies to decommission ageing offshore oil and gas infrastructure. Two main decommissioning options exist: 1) full removal of subsurface and surface facilities for terrestrial waste management or 2) abandonment in situ of assets such as subsea pipelines, moorings, platform jackets. The latter option may provide substantial environmental benefits, including the provision of artificial reef-like structures, that may support commercial benefits through fisheries. However, non-hydrocarbon contaminants on the internal surfaces of production infrastructure,

including mercury, have long been overlooked for in situ abandonment cases.

This presentation provides an overview of recent research in Australia to characterise mercury contamination in subsea gas pipelines (i.e., determine mercury speciation and seawater solubility) and assessments of potential impacts to the marine environment if contaminated infrastructure is left in situ. Examples of using mercury radioisotope tracers to determine mercury bioaccumulation by marine organisms through simplified food webs will be discussed, as well as areas for future research into the long-term fate of mercury in the marine environment from this sector

Track 4. Ecological and Human Health Risk Assessment of Chemicals, Mixtures and Stressors and Risk Mitigation Strategies

4.01.P One Health: Contributions From Environmental Toxicology and Chemistry to This Global Initiative

4.01.P-We315 Metals and Metalloids in Sediment and Aquatic Organisms: Implications for Human Health in Ecuadorian Mangrove Forests

Karla Ajoy¹, Frank von Hippel¹, Katuska Paola Calle Delgado², Genesis Portilla², Omar Alvarado² and Carlos Cajamarca², (1)University of Arizona, United States, (2)ESPOL, Ecuador

Mangrove reserves in the Gulf of Guayaquil, Ecuador, are under threat from industrial, agricultural, and aquacultural activities that introduce pollutants into these coastal ecosystems. This study aims to evaluate the concentrations of toxic metals and metalloids in mangrove environments as indicators of overall pollution levels. We collected sediment, fish, and mussels from two reserves: Reserva de Producción Faunística Manglares El Salado (RPFMS) and Refugio de Vida Silvestre Manglares El Morro (REVISMEM). Dietary intake surveys were conducted with residents of Puerto Hondo to perform a human health risk assessment that analyzes the impact on local fishermen. Our analysis revealed that iron (Fe) and copper (Cu) concentrations in sediment samples exceeded USEPA standards. In fish samples, arsenic (As), lead (Pb), and mercury (Hg) levels surpassed USEPA standards, while chromium (Cr), manganese (Mn), and zinc (Zn) levels exceeded FAO guidelines. For mussel samples, only zinc (Zn) levels exceeded both FAO and WHO guidelines. To analyze the data, we normalized and logarithmically transformed the results to conduct a Principal Component Analysis (PCA), which identified the main influencing elements in the sediment, fish, and mussels. This project aims to enhance understanding of the ecological effects of pollutants on these diverse ecosystems, providing a scientific basis to prioritize conservation efforts and inform public policy decisions.

4.01.P-We316 Operational Invertebrate Behaviour Videotracking for Real-Time Wastewater Micropollutant Surveillance

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As water resources face increasing scarcity, contaminants of emerging concern (CECs) are becoming more prevalent in aquatic systems. Traditional monitoring approaches at wastewater treatment plants (WWTPs) rely heavily on periodic grab sampling and limited bioassays, which are insufficient to capture the complex and dynamic behavior of micropollutants and their temporal discharges. This gap limits our understanding of CEC transfer to and impact on aquatic ecosystems, posing significant challenges to environmental and public health under the One Health framework.

This study introduces an innovative, real-time biomonitoring approach using the multispecies device ToxMate, leveraging avoidance behavior in aquatic invertebrates as a biomarker to characterize micropollution in WWTP effluents. By integrating laboratory and on-site monitoring, we aim to bridge the divide between chemical analysis and ecological relevance.

Our findings involve 1/ Real-World Monitoring: Long-term biomonitoring at urban WWTPs reveals episodic contamination events in effluents, identified through bioactivity signal surges. These events highlight moments of unsuitability for water reuse, alert operators to undetected pollutants, and improve operational efficiency. This real-time monitoring provides critical insights into micropollutant transfer and ecological impact, with implications for optimizing wastewater reuse strategies and safeguarding aquatic ecosystems. 2/ Data-Driven Insights: Laboratory testing of over 40 micropollutants including pesticides, polycyclic aromatic hydrocarbons (PAHs), and pharmaceuticals produced a robust behavioral database through repeated sublethal exposure. Using functional data analysis (FDA), we modeled time series data to develop behavioral fingerprints, enabling multivariate classification techniques such as principal component analysis and supervised learning. This framework enhances the identification of unknown and persistent CECs in real-time alerting systems.

This work underscores the potential of aquatic species as sentinel indicators in wastewater monitoring and exemplifies the One Health vision by connecting environmental biomonitoring to broader public health and ecosystem sustainability objectives. The proposed approach demonstrates how effect-based

biomonitoring can complement chemical analysis, improving both detection and mitigation of micropollutants in aquatic environments.

4.01.P-We317 The SPRINT Project - Sustainable Plant Protection Transition: A Global Health Approach

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SPRINT will develop and validate a Global Health Risk Assessment Toolbox to integrate assessments of the impacts of plant protection products (PPP) on ecosystem, plant, animal and human (EPAH) health, using three main attributes for health status: resilience, reproduction/productivity and manifestation of diseases. The goal is integrated risk assessment at the local, regional, national and European level, focusing on different PPP use patterns and detected residue mixtures in contrasting farming systems (conventional, integrated, organic). SPRINT consists of 9 interlinked work packages. The distribution and the impacts of PPP on EPAH health will be evaluated at 11 case study sites (CSS), ten located in diverse agricultural European landscapes, and one in Argentina (soy production for feed for EU market). PPP environmental pathways, and direct (food/feed ingestion) and indirect (air/dust inhalation and dermal uptake) animal and human exposure routes will be assessed to improve current fate, exposure, and toxicokinetic models (e.g. EFSA-FOCUS, BROWSE, BREAM). (Eco)toxicological assays will be performed based on CCS findings, using existing and improved procedures, including alternative testing criteria and new target organisms. Such assays will cover direct and indirect exposure to multiple PPP residues, realistic ranges of PPP concentrations, multi-species scenarios, and short- and long-term time horizons. Modelling of sustainability and cost-benefit analysis at the farm and macroeconomic level will be conducted to derive recommendations for sustainable transition pathways, and a research agenda on PPPs. SPRINT is based on a multi-actor approach with CCS platforms to engage stakeholders and identify respective needs, collaboration along relevant WPs, improving farmer and citizen awareness, joint development of novel management strategies for reduced reliance on PPP use, and creation of an enabling environment for adoption and change.

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4.01.P-We318 One Health Approach to Addressing Environmental Pollution: Learnings from the Environmental Pollution Programme in South Africa

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Environmental pollution, along with climate change and biodiversity loss, represents the triple planetary crisis with profound implications for human, animal, and environmental health. These interconnected challenges disproportionately impact vulnerable populations, particularly in low- and middle-income countries (LMICs), where pollution-related health risks are most acute. This motivated the UK's Department for Environment, Food and Rural Affairs (Defra) and the Joint Nature Conservation Committee (JNCC) to collaborate with partners in South Africa on an Environmental Pollution Programme, funded by Official Development Assistance (ODA). The programme embodies a One Health approach by recognising the interdependence of human, animal, and environmental health in tackling pollution. The presentation will reflect on lessons learned from the implementation of ten research projects in South Africa, developed through a collaborative effort involving local communities, scientists, practitioners, and government agencies. These projects span a range of innovative approaches, from nature-based solutions for the protection of springs, to community awareness campaigns on the impact of solid waste, to investigating pharmaceutical pollution risks in freshwater ecosystems. By engaging diverse actors and working at various scales, the programme has underscored the critical interlinkages between pollution and the health of humans, animals, plants, and the broader environment. The presentation will highlight the importance of community-driven solutions, the integration of scientific research with local knowledge, and the vital role of cross-sector collaboration in tackling the complex challenges of environmental pollution, climate change, and biodiversity loss. Insights gained from these projects are relevant for advancing the One Health agenda in LMICs and for informing strategies towards addressing the broader planetary health crisis.

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4.01.P-We319 Life Cycle Tests to Unravel Reproductive and Developmental Effects in *C. elegans* **Fabio Campos, Maria D. Pavlaki, Amadeu M.V.M. Soares and Susana Loureiro, Centre for**

Environmental and Marine Studies (CESAM) & Department of Biology, University of Aveiro, Portugal

Chemical substances from various sources enter wastewater treatment plants and end up in the environment. Pesticides and pharmaceuticals are usually some of the most found chemical substance classes found in waterbodies and soil. The ubiquitous distribution of these substances raises worldwide concern regarding their potential deleterious effects on non-target organisms and humans. Adverse effects in reproduction output are some of the most concerning ones, where some effort is needed to accurately pinpoint process where the disruption occurs. In order to provide some insights, *Caenorhabditis elegans* figures out as an outstanding animal model to tackle this problem due to its well-known lifecycle and effectively suitable within a One Health approach.

In this study, three chemical substances, found worldwide in waterbodies, were selected: the neurotoxic insecticide cypermethrin, the herbicide MCPA, and the over-the-counter pharmaceutical diclofenac. Different life stages of *C. elegans* life cycle were assessed for each substance to unravel the effects in distinct reproductive stages/processes. The embryotoxicity/neonatal toxicity was evaluated by assessing the hatching rate of exposed *C. elegans* eggs; the developmental toxicity was evaluated by exposing individuals from L1 larvae to egg-laying adults and assessing the total reared offspring; the transgenerational effects (mother-to-progeny) were evaluated by measuring the hatching rate of unexposed eggs obtained from exposed adults. Our results show that all substances affect the reproductive output of *C. elegans* by exerting effects in one or more life stages/reproductive processes. Overall, and surprisingly, the herbicide MCPA was considered as the most toxic tested substance due to the significant observed embryogenic, developmental and transgenerational effects.

The present study's targeted approach provides supporting evidence on the unexpected reproductive effects of chemicals unrelated to their mode-of-action. The results obtained also highlight the potential of *C. elegans* to fill in some gaps in the development of Adverse Outcome Pathways contributing to a more realistic and reliable hazard prediction which can be extended to other organisms and, ultimately, humans, within the One Health approach.

4.01.P-We320 Ecotoxicological Effects of Copper and Pendimethalin at Three Different Temperatures on *Danio rerio* - The Impacts of Climate Changes in One Health World

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Nowadays, the effects of climatic change have been associated with extreme weather events which impact various activities and ecosystems. The exponential increase in world population and the growth in food consumption are placing unprecedented demands on agriculture and natural resources, leading to an intensive use of fertilizers and pesticides to meet food production requirements. Agriculture remains one of the major players in the degradation of aquatic systems. Copper is widely used in biological agriculture practices and is a component of several other pesticides. Copper belongs to the group of transitional essential metals, of vital importance for every organism at low concentrations, becoming toxic at high amounts. Pendimethalin is a widely used herbicide part of the dinitroaniline family. It is used to control annual grass and broadleaf weeds in various crops and residential lawns. These compounds may put at risk the One Health initiative. Thus, this study aims to determine the combined toxic effect of a range of temperatures (15 °C, 20 °C and 25 °C) and two pesticides (Pendimethalin and Copper) on the embryonic development of Zebrafish (*Danio rerio*). Both compounds were tested using the Fish Embryo acute toxicity Test. At different temperatures, the behavioral impact of the compounds was tested at the end of the tests using a ZebraBox to assess locomotory activity. Embryos were individually exposed in flat-bottom 24-well plates. Low lethality did not allow to assess lethal concentrations. However, it was assessed the effect concentration for hatching (copper) and pericardial edema (pendimethalin).

Locomotory activity was significantly impacted by all concentrations of copper at both temperatures. In the case of pendimethalin only the highest concentration impacted the locomotory activity of the exposed embryos. Assays deployed at 15°C could not be completed for any of the compounds since embryos did not develop due to low temperature, dying after 48 hours. In conclusion, both compounds are toxic to *D. rerio* embryos; however copper is more toxic than pendimethalin. Moreover, our data point to lower

toxicity with the increase of temperature for both compounds. In conclusion, both compounds are toxic to *D. rerio* embryos, with copper being more toxic than pendimethalin. Our data indicate that toxicity decreases with increasing temperature. From One Health perspective, it is crucial to study the effects of these contaminants on aquatic life.

4.01.P-We321 Alternative Assays for Routine Toxicity Assessment

Wouter Lanneau¹ and **Kirit Wadhia²**, (1)Microbiotests Inc., Belgium, (2)NOV, United Kingdom

A substantial number of aquatic toxicity tests exist and are deployed in particular frameworks. However, for routine screening of environmental samples and potential chemical formulation investigations, these conventional assays are not practical and hinder expeditious progress. The development of alternative small-scale bioassays referred to as microbiotests has significantly advanced throughput strategies. The microbiotest technology has several advantages in comparison to the traditional tests based on laboratory cultures, especially its independence of the stock culturing burden.

The Daptoxkit is a prime example of a microbiotest that has been effective and has enabled implementation of projects worldwide otherwise not doable and has accelerated the advancement of research & development programs. Microbiotests offer new opportunities for effects monitoring of chemicals and environmental matrices (effluents, leachates, interstitial waters, etc). These can also contribute to increasing the cost efficiency and diagnostic potential of hazard assessment schemes. Use of *Daphnia* microbiotests is continuing to gather momentum for application in regulatory frameworks. Aquatic microbiotests like Daptoxkit, invaluable for screening and ranking ecotoxic effects, their intrinsic features can contribute to novel applications enabling rapid detection and enhanced throughput. Biological testing, and microbiotesting in particular, will indubitably increase significantly in environmental protection activities in the future. International recognition of biotesting, and more critically owing to limitations in regional testing infrastructure (lack of laboratories and culturing facilities), the implementation scope is globally significantly enhanced to facilitate environmental policies, regulations and guidelines. The imperative need for cost efficiency in environmental assessment continues to fuel the need for R & D concerning microbiotesting.

4.01.P-We322 Of Pigs and Men – Comparing PFAS Toxic Load Patterns in Wild Boar Liver and Human Blood Samples

Tobias Frische, German Environment Agency (UBA), Section II 2.6 Soil Protection Measures, Germany

Diffuse PFAS contamination of the environment across the globe has become increasingly evident in the very past years and due to the toxic potential of several well-studied PFAS compounds (e.g. PFOA, PFOS) gives rise to intense scientific attention and public concern. Hence, uptake by and accumulation of PFAS in biota from contaminated environmental media and subsequent food-chain transfer may cause severe long-term adverse effects on human and wildlife populations. Against this background, a comparative data analysis was conducted to explore similarities and differences in PFAS internal exposure and toxic load patterns between humans and wild boars (*Sus scrofa* L.) both being (by origin) omnivorous terrestrial mammals with however clearly different potential PFAS exposure pathways. First, chemical patterns of the most dominating PFAS measured in human blood and wild boar liver tissue samples were generated from already published data. The analysis focused on samples that are assumed to reflect diffuse background PFAS contamination as well as on samples obtained from a large-scale PFAS hot spot area known for severe contamination of soil and groundwater. Additionally, PFOA equivalents were calculated as toxicological descriptors using the relative potency factor (RPF) approach: The respective content of each of the PFAS targets is scaled relative to the index compound PFOA with subsequent summation of the individual PFOA equivalents yielding a measure of the total toxic load, and the relative contribution (%) of individual PFAS compounds to the summed PFOA equivalents illustrating the toxic load pattern. Long-chain PFAS do accumulate in wild boar livers with following decreasing rank order in relative contribution to the overall toxic load (Frische et al., 2023): PFOS >> PFNA > PFDA > PFUnDA = PFDoDA = PFTrDA; toxic load patterns observed in wild boar liver samples collected in background PFAS contamination areas differ from sample cohorts nearer to the PFAS hot spot. The respective data analysis for human blood samples is still underway, the observed differences of chemical and toxic load patterns between wild boar and human samples will be discussed in the presentation.

4.01.P-We323 Effects of Bisphenols to Amphibians and Fish: An Integrative Approach

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Plastic contamination poses a major environmental issue, primarily due to improper disposal and landfill runoff, which have led to the ubiquitous presence of plastics in aquatic environments. Alongside plastics themselves, intentionally added substances like plasticizers and flame-retardants can leach into the surrounding environment as they are not covalently bound to the plastic matrix. Characterizing the hazards of these substances, especially plasticizers such as bisphenol A (BPA) analogues, is critical. These analogues may be as toxic or even more toxic than BPA, and their environmental presence is expected to increase due to the demand for "BPA-free" in commercialized products and BPA restrictions in food-contact materials. This study aimed at a comparative assessment of the toxicity of BPA and bisphenol AF (BPAF) for amphibian and fish models, namely for: *Xenopus laevis* cell lines, embryos and tadpoles, and for *Danio rerio* embryos and larvae. To attain this objective, 72-h cytotoxic assays were conducted with the *X. laevis* A6 cell line, where cell viability was assessed through the MTT viability assay. Embryos of *X. laevis* and *D. rerio* were exposed for 96-h and 120-h (respectively) to assess mortality, occurrence of malformations and heart rate. Additionally, tadpoles and zebrafish larvae were exposed to sublethal concentrations for 48-h to analyse key components for locomotor behaviour with an automated tracking system. Obtained results suggest that BPAF was more toxic to both *X. laevis* embryos and A6 cell line than BPA. Moreover, *D. rerio* embryos also exhibited a similar trend, where BPAF presented a lower 120h-EC50 (mortality and malformations) than BPA. Furthermore, BPAF also showed to highly affect heart rate compared to BPA for *D. rerio* embryos suggesting higher toxicity of this BPA analogue for this species life stage. These findings highlight the need for an extensive assessment on bisphenol analogues, like BPAF which exhibits higher toxicity compared to BPA. The increased use of BPA-free products in response to growing concerns to BPA's environmental impacts may inadvertently lead to a greater exposure to these potentially more harmful analogues. This study emphasizes the importance to rethink material safety standards and the need for a robust and comprehensive risk assessment of all bisphenol derivatives, given their widespread use and applications of these compounds, potentially harming both human and environmental health.

4.02 Addressing Regulatory Gaps in Polymer Risk Assessment: Advances in Testing, Novel Test Methods, and Environmental Impact Evaluations

4.02.T-01 Leveraging Increased Microbial Cell Concentrations to Assess the Biodegradability of Water Soluble and Water-Dispersible Polymers in Laboratory Tests

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Water-soluble and water-dispersible polymers are essential ingredients in home and personal care formulations, offering unique benefits in terms of performance and sustainability. For polymeric materials, regulations world-wide are rapidly developing with greater focus on the assessment of their degradability in applications leading to environmental exposure. This shift emphasizes the need to identify and critically evaluate testing guidelines for assessing biodegradability of polymeric materials.

OECD test guidelines were developed to assess the biodegradability of discrete low molecular weight, water soluble substances, making it essential to validate, adapt or develop new protocols for polymers. In contrast to low molecular weight substances, and owing to their macromolecular nature, polymers require an extracellular enzymatic cleavage before the degradation products can be assimilated by microbes for metabolism. This extra step may result in intrinsically longer times for degradation and a disproportionately larger number of polymers that fail to meet the passing thresholds associated with OECD screening tests. In addition, the presence and activity of extracellular enzymes and microbes producing them are critical for assessing polymer biodegradability in laboratory methods.

In this study, we investigate in an interlaboratory effort the potential of modifying screening biodegradation tests by increasing the concentration of inoculum (sludge) to assess the biodegradation of

water-soluble polymers. We explore various polymers relevant for home and personal care formulations with differing chemistries, molecular weights, and degrees of substitution, including polyethylene glycol, carboxymethyl cellulose, cationically modified guar gum, and polyvinyl alcohol. A direct comparison is made between standard OECD 301 B and F testing protocols and modified conditions with increased microbial abundance (both concentration and in totality via increased test volumes) to test chemical ratios. For both protocols, testing times have been extended to reach biodegradation plateau.

Our investigations highlight the potential of methods leveraging increased cell concentrations and extended test times for more accurate and reliable determinations of the biodegradability of water-soluble polymers. Furthermore, our findings highlight strengths and limitations of existing OECD 301 test methods to investigate water-soluble polymers.

4.02.T-02 Moving Biodegradation Testing of Water-Soluble Polymers Closer to Environmentally Realistic Scenarios

Aaron Kintzi¹, Soumya Daturpalli², Glauco Battagliarin³ and Michael Zumstein¹, (1)University of Vienna, Centre for Microbiology and Environmental Systems Science, Division of Environmental Geosciences, Austria, (2)BASF SE, United States, (3)BASF SE, Limburgerhof, Germany

Water-soluble polymers (WSPs) are essential for the performance of many home and personal care products. Being disposed down-the-drain after use in many applications, biodegradability in wastewater systems is a desirable feature of sustainable WSPs. To support the development and regulation of biodegradable WSPs, scientifically valid and economically viable biodegradation test methods are essential. Existing biodegradation testing guidelines, such as the OECD 301 series, were developed for small molecules, warranting a critical assessment of their applicability to WSPs, which we aim to contribute to through this study.

In brief, we explored potential test modifications by evaluating the impact of individual and combined protocol variations of the OECD 301 guidelines on the biodegradation of WSPs by wastewater microbiomes. We incubated a selected set of WSPs (e.g., the polyamino acids polylysine and polyaspartic acid, polyethylene glycol, and polyvinyl alcohol) and low-molecular-weight reference substrates with wastewater microbiomes, quantified biodegradation with respirometric methods, and conducted complementary enzyme activity assays and microbial community analyses.

Key protocol variations involved (i) washing and aerating microbial inoculum before WSP addition, (ii) pre-incubating WSPs with filter-sterilized untreated wastewater, and (iii) examining the effects of WSP concentration. Most importantly, combined protocol variations showed substantially accelerated biodegradation of the tested poly(amino acids) compared to the standard protocol. As these variations did not significantly affect the biodegradation of low-molecular-weight reference compounds, our findings indicate that the observed effects are specific to polymers. Building on these results, we are currently working with complementary methods to further move laboratory-based WSP biodegradation testing towards realistic scenarios. Together, our contribution reveals promising biodegradation data for poly(amino acid)s and highlights challenges and opportunities for WSP biodegradation testing.

4.02.T-03 Phys-Chem Challenges in Regulatory: Water Solubility and Octanol/Water Partition Coefficient

Joachim Venzmer, Evonik Operations GmbH, Research Interfacial Technology, Germany

Some methods which were originally developed for low molecular weight, non-amphiphilic molecules are hardly applicable to surfactants and amphiphilic polymers. This results in highly questionable values, but if they are below some threshold, they did not raise any alarm and were accepted without questioning their validity. As long as the results do not cause any negative consequences, this could be ignored but what to do if this was not the case?

Even supposedly easy parameters such as solubility in water (OECD TG 105) are challenging: For surfactants, (A) there is no saturation concentration, and (B) above the so-called Critical Micelle Concentration (CMC), they form micelles of few nm in size. Unfortunately, there is a terrible misconception: Micelles have been described as not bioavailable, insoluble hydrophobic droplets of surfactants completely ignoring the rapid (timescale μ s to ms) exchange between the monomers in the bulk phase and the molecules within the micelles. Therefore, the factual solubility of surfactants as aggregates is orders of magnitude higher than the CMC.

The octanol/water partition coefficient $\log K_{ow}$ is a measure of hydrophobicity/hydrophilicity which has also never been meant to be used for surface-active materials; it is used to predict the (eco)toxicological consequences. One accepted option is a calculated value based on CMC and the solubility in octanol. The latter is determined by OECD TG 105 and replacing water by octanol. Since the method is based on visual observation, the determination of the solubilities in octanol leads to results which are several orders of

magnitude overestimated: Coarse emulsions look crystal clear and are mistaken as solubility, because the difference in refractive index between octanol and the sample is often rather low. As a consequence, these values for logKow are much too high and do not reflect the properties of the substance tested. Phys-chem data such as solubility and logKow should be a measure of the properties of a substance tested. If, however, the methods to be used are hardly applicable to surface-active materials, there should be an agreement to which extent science should prevail over taking non-applicable technical guidelines literally. These three most pressing issues are currently being addressed by the CESIO Working Group Test Methods of Surfactants (TMS) and the Phys-Chem Workstream of the recently established ECETOC Polymer Analytics Task Force.

4.02.T-04 Anticipating the Implementation of Polymer REACh: Exploring the Technical Challenges for Polymers in OECD Test Guidelines for Solubility and (Eco-)Toxicity Tests

Simon Luderwald¹, Friederike Luenne², Denis Botin², Nora Hartner², Naveed Honarvar¹, Patrick Baudy-Groh³ and Susanne Noreen Kolle¹, (1)BASF SE, Experimental Toxicology and Ecology, Germany, (2)BASF SE, Analytical and Material Science, Germany, (3)Experimental Toxicology and Ecology, BASF SE, Germany

As part of the European Green Deal and the Chemical Strategy for Sustainability, it is anticipated that REACH registration will be expanded to include polymers, incorporating data requirements for analytical, ecotoxicological, and toxicological assessments, primarily based on established OECD test guidelines (TGs). These TGs have been validated for small molecules, and their technical applicability and predictive capabilities have not been thoroughly assessed across the entire spectrum of chemical substances, especially polymers were not included. Unlike small molecules, polymers are inherently characterized by e.g. distribution of chain lengths, different end-groups, and unique architectures. Therefore, the technical applicability of the existing TGs on polymers is potentially limited and involves the risk of generating unreliable data impacting risk assessments. In our study, we evaluated the technical applicability of several OECD TGs using a set of 17 different polymers. The determination of solubility and extractability was based on OECD TG 120 for polymers, however, the sample preparation procedures outlined are not universally applicable to all polymer types. Our assessment also included various test systems determining (eco-)toxicological effects: OECD TG 202 (*Daphnia* sp. acute immobilization test), OECD TG 487 (in vitro mammalian cell micronucleus test), and OECD TG 439 (in vitro skin irritation: reconstructed human epidermis test method).

The development of standardized methods for analytical, ecotoxicological, and toxicological evaluations is crucial, especially for complex materials like polymers. In this context, we highlight the challenges of applying existing OECD test guidelines to polymers and propose alternative approaches that may be more appropriate.

4.02.P-We324 Navigating the Limitations of SEC: Strategies for Improved Polymer Analysis in Regulatory Frameworks

Timo F. Beskers¹ and Jana Falkenhagen², (1)BASF SE, Germany, (2)BAM Bundesanstalt für Materialforschung und -prüfung, Germany

Polymers, composed of repeating units called monomers, exhibit a diverse range of properties, with molecular weight distribution (MWD) being particularly important for regulatory risk assessments. Size exclusion chromatography (SEC), or gel permeation chromatography (GPC), is the primary technique for MWD characterization, as detailed in OECD guidelines 118 and 119, along with DIN and ISO standards. However, SEC faces significant shortcomings that complicate the accurate determination of MWD and oligomer content. These limitations include the insolubility of certain polymers, crosslinking, and ultra-high molecular weight characteristics, which can exceed SEC's separation capabilities. The presence of salts or modifiers can further interfere with oligomer content determination, and the availability of appropriate SEC columns and calibration standards limits comparability across laboratories.

To enhance SEC's reliability and applicability within regulatory frameworks, innovative methods and strategies are essential. Proposed solutions involve new sample preparation techniques, advanced data interpretation methods, and the establishment of standardized methodologies to promote harmonization. This is especially crucial in light of the expected Polymer REACh (PREACh) legislation, which aims to regulate polymer registration and risk assessment within the European market.

Given the anticipated complexity of the registration process, which requires extensive data generation, proactive strategies are needed to address SEC's challenges. Currently, there are no viable alternatives for oligomer content determination, and the unique properties of polymers often necessitate tailored analytical approaches. Without new methodologies and guidelines, the polymer registration process could become burdensome and costly, jeopardizing important products and markets in the EU.

Key areas for improvement include extending existing norms to cover a broader range of solvents,

addressing high error margins in oligomer content determination, establishing harmonized approaches for dealing with side components, and providing clarity on measuring partially soluble polymers. In conclusion, addressing SEC's limitations through the development of comprehensive guidelines and harmonization is critical for ensuring accurate and efficient polymer analysis, ultimately facilitating smoother registration processes.

4.02.P-We330 Next-Generation Biodegradation Testing for the PLF Market: Re-envisioning Biodegradation Standards Tests

Nick W. Johnson¹, Sam B.H. Patterson², Trevor Hinchcliffe², Filipe Vilela¹ and Humphrey H.P. Yiu¹, (1)Heriot-Watt University, United Kingdom, (2)Impact Solutions, United Kingdom

Polymers in liquid formulation (PLFs) represent a significant proportion of the polymer market (\$125.2 billion) and are vital to a diverse range of industries including personal care, paints and agriculture. The addition of PLFs are required to alter the chemical and rheological properties of liquid formulation products to improve longevity, resistance to degradation, and overall product performance. However, due to a change in current EU REACH regulations, Article 138 (2), requiring the registration of polymeric substances, there is a requirement to replace existing PLFs with more sustainable, biodegradable alternatives in a market which is profoundly reliant on petrochemical feedstocks. However, the development of novel biodegradable polymers is hindered by existing biodegradation standard testing, which is time consuming, not fit-for-purpose and does not provide the data required by the industry. To address this concern, Impact Solutions are developing advanced solutions for biodegradation testing aimed at the PLF market. Initially, we are developing a colorimetric assay to assist industry in the preliminary development stage. This 96-well plate assay works in line with current OECD standard biodegradation testing and will provide a high-throughput in-house method to screen vast numbers of potential biodegradable polymers. Additionally, in a collaboration with Heriot-Watt University, we are looking to build on previous work on the enzymatic depolymerisation of polycaprolactone to conduct biodegradation studies on a fluidised bed reactor. By providing continuous data analysis, bespoke advanced datasets can be generated and customised to assess how the polymer is broken down in the environment. These datasets, which are currently not accessible using current standard tests, could be subsequently employed to provide vital information on the environmental fate and ecotoxicity of a polymer and potentially pave the way to generate biodegradation simulation models in line with models which are currently available to small molecules.

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4.02.P-We335 Innovating on Polymer Degradation: Making Two Ends Meet

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Polymers play a pivotal role in modern society by providing a multitude of technical functionalities in materials and products. They represent a vast and diverse aspect of the chemical space, often characterized by medium to very high molecular sizes and various functionalities. For polymers emitted down the drain, ultimate degradability becomes a favourable property. Yet, assessing the environmental fate of polymers poses scientific challenges, primarily due to their high molecular weight, which leads to low bioavailability impacting for example uptake in biodegradation studies.

In line with their emission routes, polymers used in Home and Personal Care products are evaluated for their biodegradation potential. However, the domain of applicability of standardized test guidelines, such as those from the OECD, does not include polymers. Therefore, the innovation of new materials faces two main challenges. The first is the need to accelerate the development of screening methods to inform product development, while the second is aligned to the need for the adaptation of standardized test methods to widen the applicability domain to polymers. In this presentation, we delve into methodologies for evaluating the biodegradation of polymers in aqueous systems during product development by use of screening and modified test methods.

At early stages of development, combining enzymatic assays with analytical techniques like ¹H NMR spectroscopy enables the characterization of changes in structural features. Additionally, candidate polymers are evaluated using standard or modified OECD biodegradation screenings studies. Adaptions including the extension of the test durations, are valuable approaches in comparing the biodegradation polymers with similar structural properties.

4.02.P Addressing Regulatory Gaps in Polymer Risk Assessment: Advances in Testing, Novel Test Methods, and Environmental Impact Evaluations

4.02.P-We324 Navigating the Limitations of SEC: Strategies for Improved Polymer Analysis in Regulatory Frameworks

Timo F. Beskers¹ and Jana Falkenhagen², (1)BASF SE, Germany, (2)BAM Bundesanstalt für Materialforschung und -prüfung, Germany

Polymers, composed of repeating units called monomers, exhibit a diverse range of properties, with molecular weight distribution (MWD) being particularly important for regulatory risk assessments. Size exclusion chromatography (SEC), or gel permeation chromatography (GPC), is the primary technique for MWD characterization, as detailed in OECD guidelines 118 and 119, along with DIN and ISO standards. However, SEC faces significant shortcomings that complicate the accurate determination of MWD and oligomer content. These limitations include the insolubility of certain polymers, crosslinking, and ultra-high molecular weight characteristics, which can exceed SEC's separation capabilities. The presence of salts or modifiers can further interfere with oligomer content determination, and the availability of appropriate SEC columns and calibration standards limits comparability across laboratories.

To enhance SEC's reliability and applicability within regulatory frameworks, innovative methods and strategies are essential. Proposed solutions involve new sample preparation techniques, advanced data interpretation methods, and the establishment of standardized methodologies to promote harmonization. This is especially crucial in light of the expected Polymer REACH (PREACH) legislation, which aims to regulate polymer registration and risk assessment within the European market.

Given the anticipated complexity of the registration process, which requires extensive data generation, proactive strategies are needed to address SEC's challenges. Currently, there are no viable alternatives for oligomer content determination, and the unique properties of polymers often necessitate tailored analytical approaches. Without new methodologies and guidelines, the polymer registration process could become burdensome and costly, jeopardizing important products and markets in the EU.

Key areas for improvement include extending existing norms to cover a broader range of solvents, addressing high error margins in oligomer content determination, establishing harmonized approaches for dealing with side components, and providing clarity on measuring partially soluble polymers. In conclusion, addressing SEC's limitations through the development of comprehensive guidelines and harmonization is critical for ensuring accurate and efficient polymer analysis, ultimately facilitating smoother registration processes.

4.02.P-We325 Error Margins in the Determination of Molar Mass and Oligomer Content of Polymers

Jana Falkenhagen¹, Jan-Hendrik Arndt², Timo F. Beskers³, Alexander Eifert⁴, Sabine Gaul⁵, Sabine Keune⁶, Ron A.H. Peters⁷, Sebastian Schmiedt⁸ and Alexander von Tesmar⁹, (1)BAM Bundesanstalt für Materialforschung und -prüfung, Germany, (2)Fraunhofer LBF, Germany, (3)BASF SE, Germany, (4)Wacker Chemie AG, Germany, (5)Clariant Innovation Center, Germany, (6)Evonik Operations GmbH, Germany, (7)Uni Amsterdam, Van 't Hoff Institute for Molecular Sciences, Netherlands, (8)Eurofins Agroscience Services EAG Laboratories GmbH, Germany, (9)Currenta GmbH & Co. OHG, Germany

Size exclusion chromatography (SEC) is still the method of choice for determining the molar mass and molar mass distribution of macromolecules.

It is a liquid chromatographic technique that separates molecules based on their size, respectively their hydrodynamic volume using a porous stationary phase that allows smaller molecules to pass through the pores while excluding larger molecules. The chromatogram from the size separation can be calibrated using polymers of known molar mass resulting in a relative molar mass distribution curve. From this distribution curve molar mass averages and the oligomer content (percentage of peak area smaller than 1000 g/mol and 500 g/mol) of polymers can be derived.

The determination of oligomer content is important, because it is often used in regulatory contexts as a measure of small, mobile and potentially more toxic parts of the polymer. Polymer regulations are of increasing political and social importance. Regulations often set very narrow limits.

SEC has rather large error margins of 10-20% for the molar mass determination performed in different laboratories or on different instruments. These variances are well known to all experts and users and have been investigated in several round robin tests in the past. These resulted in DIN and ISO standards which, in our opinion, no longer meet today's requirements.

As far as we know, the margins of error in determining oligomer content have not yet been investigated. It is expected that the variation will be rather high. This is because it depends on many factors related to the experimental conditions (column sets used, solvent, temperatures, sample preparation) on the one hand, and the data evaluation mechanisms (choice of baseline and peak limits) on the other.

In order to actually measure the error margins and to support this with actual data a round robin test has been performed from June to November 2024 with approx. 40 participating laboratories. Three different solvents were selected, namely THF, DMAc or DMF and water. The samples were selected together with all the experts, taking into account a wide range of practical aspects far from narrowly distributed standards: e.g higher distribution range, reduced solubility, included side components, copolymers and dispersions with gel content. In this contribution we will present the detailed concept of this round robin test and first results.

4.02.P-We326 An Algorithm to Address Side Components in SEC Chromatograms of Polymers for Regulatory Purposes

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Size Exclusion Chromatography (SEC) is a crucial method for analyzing the molecular weight distribution (MWD) and oligomer content in polymers. Accurately determining oligomer content is vital for regulatory risk assessments concerning polymers and is a fundamental requirement for polymer substance registration in various countries, including the anticipated revisions to REACH in the EU. SEC operates by separating molecules according to their size in solution, specifically their hydrodynamic volume, which makes it an entropic process that is not specific to any chemical characteristics.

However, the presence of side components in the sample presents significant challenges for SEC analysis. To achieve accurate results, SEC measurement conditions must be optimized for the specific polymer, requiring a careful balance of polarity among the sample, solvent, and stationary phase. This is essential to avoid enthalpic interactions that could disrupt the SEC separation mechanism. Side components can vary in chemical nature and polarity; if they share similar polarities with the polymer, they may elute within the calibrated SEC range, leading to overlap with polymer signals and complicating the determination of oligomer content and MWD. Side components with differing polarities may elute outside the SEC chromatogram or may go undetected if they are insoluble in the solvent.

Consequently, the oligomer content derived from SEC cannot fully encompass all low molecular weight species present, nor does it consistently isolate the polymer oligomer content due to its lack of specificity. Analyzing real-world polymer products, which often contain impurities, is particularly challenging due to overlapping peaks. If the concentration of a side component is known and measurable, peak subtraction can provide a viable solution for analysis. Determining the exact concentration is often challenging and time consuming.

Here we will present a newly developed algorithm, which enables rapid and straightforward estimation of SEC results for the pure polymer in a mixture when only the SEC chromatograms of both the polymer with side component and the side component alone are available. This provides a fast and cost-effective solution to decide if a regulatory limit is met, even when side components are present.

4.02.P-We327 How Applicable are Existing Methods for the Determination of Adsorption/Desorption of Polymers – Application and Comparison of Test Guidelines OECD 106 and OECD 121

Sebastian Schmiedt and Paul Bendig, Eurofins Agroscience Services EAG Laboratories GmbH, Germany

The ecological hazard and risk for small molecules and products thereof is assessed among other things with the help of several physico-chemical properties. With the upcoming development and growing concerns in the use of polymers also the demand for these data for polymers is rising.

There are analytical methods described in technical guidance documents (e.g. OECD TG 100 series), which were developed for small molecules, but are not yet tested for applicability or are extended for the analysis of polymers. In general, additional research is needed to address this issue.

One of the relevant properties is the adsorption and desorption to soil or sludge, which is used e.g. to estimate the behavior in modelling the percolation to ground water or behavior in wastewater treatment plants.

The aim of the project was to compare the OECD tests 106 and 121 using well defined polymer samples in regard of applicability and possible limitations. For this purpose, the differences in the feasibility and outcome of the results are evaluated when using both test guidelines. In additions challenges such as low water solubility or analysis of high molecular weight substances could be addressed.

4.02.P-We328 Current Plastic Definitions and the Impact on Hazard Assessment of Dispersed Persistent Polymers in the Regulation of Chemicals

Malgorzata Wilczynska, James Brown, Claire Phillips, Carl Clarke, Nicola Geary, Anna Sypniewska-Huk, Nathan Read and Freya Mickleburgh, Centre for Environment, Fisheries and Aquaculture Science (Cefas), United Kingdom

Plastics are defined as solid particles which are water insoluble and are composed of mixtures of synthetic polymers and functional additives such as UV stabilizers, antioxidants, and heat stabilizers specifically intended to make them durable and persistent in the environment. The updated Europe Union (EU) regulation considers the fluidity test as one of the criteria that confirms the substance status as a plastic. Numerous polymers that are nonbiodegradable in the environment are produced in the form of colloids or gels that previously didn't fall under the plastic definition as they weren't classed as solid. The application areas for polymer colloids are diverse, including their use as binders in fabrics and paper coatings, synthetic rubbers, paints, adhesives, toughened plastics, catalytic supports, flow modifiers and resins. Global efforts to control plastics and their associated chemicals are beginning to be embraced; however, plastic colloids are not as heavily scrutinised, despite some substances being highly persistent. Those semiliquid substances can precipitate in the marine water and become entrained in the sediment of disperse or as a gel could enter the food chain.

Future actions in plastic regulations should build on existing knowledge and avoid the duplication of effort by identifying and harnessing national, regional, and global regulation.

Regulatory instruments are a major tool for controlling and phasing out chemicals of concern therefore additional review of plastic definition should be conducted in the context of the physical state and persistence of substances. Chemicals are infinitely complex and there is need for improved characterisation and tighter controls for persistent chemicals. This presentation aims to identify knowledge gaps and potential areas of concern with respect to the aquatic environment and offers solutions with respect to the application of existing regulatory frameworks for future work to reduce the impact on the environment.

4.02.P-We329 Assessing Bioavailability Water-Soluble Polymers: Conceptual and Historical Considerations

Kristin Connors and Corie Ellison, The Procter and Gamble Company, United States

Historically, water-soluble polymers have been considered of low hazard to both humans and the environment since they have a high molecular weight and are often assumed to have poor/negligible bioavailability. In the 2024 ECHA report on Key Areas of Regulatory Challenge, ECHA noted that "high molecular weight polymers can no longer be considered innocuous by default" and further emphasized the need to build a fundamental understanding of polymer bioavailability. Bioavailability is a critical consideration in both human and environmental hazard and risk assessments, including in the assessment of bioaccumulation potential. Several physiochemical properties are known to limit bioavailability including molecular weight, molecular diameter/bulkiness, lipophilicity, and ionization. In this presentation, we will provide a conceptual overview of bioavailability as it relates to relevant routes of exposure for both human and environmental hazard assessments (e.g., oral, dermal, inhalation [lungs, gills]). Experimental approaches used to characterize bioavailability will be discussed (e.g., modeling approaches, CaCo2, Ussing chambers, etc). We will summarize the historic evidence for low bioavailability of high molecular weight water-soluble polymers, highlight key uncertainties, and provide recommendations for future research needs.

4.02.P-We330 Next-Generation Biodegradation Testing for the PLF Market: Re-envisioning Biodegradation Standards Tests

Nick W. Johnson¹, Sam B. H. Patterson², Trevor Hinchcliffe², Filipe Vilela¹ and Humphrey H. P. Yiu¹, (1)Heriot-Watt University, United Kingdom, (2)Impact Solutions, United Kingdom

Polymers in liquid formulation (PLFs) represent a significant proportion of the polymer market (\$125.2 billion) and are vital to a diverse range of industries including personal care, paints and agriculture. The addition of PLFs are required to alter the chemical and rheological properties of liquid formulation products to improve longevity, resistance to degradation, and overall product performance. However, due to a change in current EU REACH regulations, Article 138 (2), requiring the registration of polymeric substances, there is a requirement to replace existing PLFs with more sustainable, biodegradable alternatives in a market which is profoundly reliant on petrochemical feedstocks. However, the development of novel biodegradable polymers is hindered by existing biodegradation standard testing, which is time consuming, not fit-for-purpose and does not provide the data required by the industry.

To address this concern, Impact Solutions are developing advanced solutions for biodegradation testing aimed at the PLF market. Initially, we are developing a colorimetric assay to assist industry in the preliminary development stage. This 96-well plate assay works in line with current OECD standard biodegradation testing and will provide a high-throughput in-house method to screen vast numbers of

potential biodegradable polymers. Additionally, in a collaboration with Heriot-Watt University, we are looking to build on previous work on the enzymatic depolymerisation of polycaprolactone to conduct biodegradation studies on a fluidised bed reactor. By providing continuous data analysis, bespoke advanced datasets can be generated and customised to assess how the polymer is broken down in the environment. These datasets, which are currently not accessible using current standard tests, could be subsequently employed to provide vital information on the environmental fate and ecotoxicity of a polymer and potentially pave the way to generate biodegradation simulation models in line with models which are currently available to small molecules.

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4.02.P-We331 Risk Assessment of Water-Soluble Cationic Polymers Reflecting Realistic Environmental Conditions

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The assessment of the ecological impacts of polymers is important due to increased regulation and public concern. Water-soluble cationic polymers used in cosmetics and personal care products have raised concerns due to their high toxicity to aquatic organisms and poor biodegradability. On the other hand, several studies suggest that cationic polymers adsorb to activated sludge at wastewater treatment plants (WWTPs) or organic matter in rivers. Therefore, the environmental concentration of cationic polymers is expected to be low and may have a small impact on the ecosystem. However, there are few studies of refined ecological risk assessments considering the removal of cationic polymers in WWTPs and their adsorption on organic matter.

In this study, exposure and hazard assessments were conducted for water-soluble cationic polymers, polyquaternium-6 (PQ-6) and PQ-7, and the risk quotients (RQs) of the polymers in Japan were determined. Daphnids and algae toxicity tests were conducted under both standard OECD Test Guideline (TG) 201 and 202 conditions and in the presence of humic acid. Subsequently, toxicity mitigation factors were calculated. The removal rate in the WWTPs was estimated by quantifying the adsorbed substances to activated sludge based on the OECD TG 314B.

The results indicated toxicity mitigation with the addition of humic acid, suggesting that the toxic effects under realistic environmental conditions are reduced compared to those observed under standard OECD test conditions. Furthermore, rapid adsorption behavior on activated sludge was confirmed, suggesting that the test substances are almost completely removed in the WWTPs. Finally, we conducted a risk assessment based on the distribution volume of these polymers within Japan. By applying the new factor obtained in this study, the RQs for PQ-6 and PQ-7 were determined to be below one, indicating no significant concern for the aquatic environment.

This study provides novel insights into the toxicity and exposure profiles of PQ-6 and PQ-7, bridging the data gaps between standard test conditions and environmentally realistic conditions. Our risk assessment of cationic polymers will further advance the discussion on the appropriate use of polymers and the risk assessment reflecting realistic environmental conditions.

4.02.P-We332 Polyvinylpyrrolidone Speeds Up and Increases Methane Production from Freshwater Sediments

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Synthetic hydrophilic polymers, such as polyvinylpyrrolidone (PVP), are extensively used for a wide range of applications, including household cleaning, personal care products, wastewater treatment, and agriculture. After their use, these polymers can enter freshwater systems. Due to their polar or charged functional groups, they dissolve easily in water, making them bioavailable to freshwater and sediment-associated organisms, potentially disrupting ecological processes. Despite these hazards, the environmental impacts of synthetic hydrophilic polymers remain largely unknown, especially for sediment-associated organisms. To address this knowledge gap, we incubated freshwater sediment under anaerobic conditions with PVP at four concentrations (0, 0.5, 50, and 5000 mg/L) for 56 days, assessing methanogenesis as a key ecosystem process. Weekly measurements of methane (CH₄) in the headspace of

the test systems showed that CH₄ production speed and total CH₄ production increased by up to 6% and 20%, respectively, at 5000 mg/L, a concentration already observed in treated wastewater. The mechanisms driving these results may be two-fold: first, exposure to PVP could have altered the bacterial community composition, potentially affecting the functional profile of methanogenic microbes. This hypothesis is currently being investigated using metabarcoding of the archaeal and bacterial 16S rRNA genes. Second, PVP itself might serve as a carbon source for sediment-associated microbial communities, increasing CH₄ production. Although the exact mechanisms remain to be uncovered, our study suggests that synthetic hydrophilic polymers can impact the environment by contributing to greenhouse gas emissions through altered methanogenesis in freshwater ecosystems.

4.02.P-We333 (Semi-)Quantitative Analysis of Polyamide 6 Oligomers in Multiple Water Samples using LC-HRMS: Evidence for the Presence of Polymer Derivatives in Drinking Water and Domestic Sewage

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Polyamide 6, a widely used polymer, can release oligomers that leach into water or migrate into food. Toxicity predictions from ProTox 3.0 suggest potential risks associated with these oligomers, for instance 1,8-diazacyclotetradecane-2,9-dione (aminohexanoate cyclic dimer, AHCD), which has an estimated LD₅₀ of 560 mg/kg and high probabilities of Blood Brain Barrier disruption (94%) and neurotoxicity (64%). Previous non-targeted analyses of drinking water tentatively identified Polyamide 6 oligomers (cyclic dimer to heptamer), motivating further investigation into their prevalence. This study optimized the target and suspect screening using liquid chromatography-high resolution mass spectrometry (LC-HRMS) to analyse six Polyamide 6 oligomers in 81 water samples, including purified drinking water (n = 59), tap water (n = 14), and sewage (n = 8). Calibration for AHCD was performed using the reference standard and internal standard (range: 0.1–200 ng/L; R² = 0.9997; LOD = 0.5 ng/L; LOQ = 1 ng/L), and recovery experiments in five replicates achieved recoveries of 89.1–126% with a relative standard deviation (RSD) of 13.0%. Predictive quantification for other Polyamide 6 oligomers using the Quantem tool was validated by spiking AHCD, showing comparable accuracy (recoveries: 86.5–123%; RSD: 13.1%). Results indicated the presence of six oligomers in purified water, with AHCD detected in 49.2% of samples (mean concentration: 86.3 ng/L; max: 828 ng/L) and total oligomer concentrations averaging 585 ng/L (max: 9,530 ng/L). The occurrence of Polyamide 6 oligomers in purified water was suggested to be associated with the use of polymer fiber wipes for the maintenance of water purifier tank. In tap water and sewage, only cyclic dimer, trimer, and tetramer were found. Tap water showed low total oligomer concentrations (max: 4.9 ng/L), whereas sewage exhibited significantly higher levels, particularly in influent from domestic sources (total concentration: 4,570 ng/L) and two fiber manufacturing facilities (total concentrations: 31,200 and 54,200 ng/L). Total oligomer concentrations declined through the sewage treatment, reaching 54 ng/L in the effluent sample. This study optimized and validated a (semi-)quantitative method for analysing Polyamide 6 oligomers in multiple water samples, providing new data to support environmental and health risk assessments for polymer derivatives.

4.02.P-We334 Elucidating the Role of Enzymes in Polymer Biodegradation: A Case Study on Poly(Aspartic Acid)

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Synthetic water-soluble polymers (WSPs) are organic molecules that dissolve, disperse, or swell in water to alter the physical properties of aqueous systems and formulations through gelation, thickening, emulsification, and stabilization. Due to their exceptional properties, they are widely used in our daily life such as in home and personal care products, cosmetics, and agrochemicals. After use, WSPs can enter into the environment, such as through wastewater treatment plants (WWTPs) or agricultural soils. However, only little information is available on their biodegradability in the environment.

Biodegradation is a multi-step process mediated via microbial metabolism. Due to their high molecular weight, WSPs must first be cleaved into smaller fragments for take up by microorganisms. This can occur abiotically or be performed by enzymes produced and secreted by microorganisms in the environment. Existing OECD biodegradation testing guidelines have been primarily developed for the testing of small

organic molecules. Nevertheless, standardized tests such as OECD 301 and 302 are currently used to assess biodegradability of polymers. However, the degradation mechanism of polymers, which necessitates break down into smaller fragments before microbial uptake and biodegradation, differs significantly from that of small molecules. As a result, existing methodologies and pass criteria may not be appropriate for assessing polymer biodegradation.

The commercially most successful synthetic poly(amino acid) is the water-soluble and anionic poly(aspartic acid). However, little is known about the biodegradability of tPAA. In this study, we investigated the interaction between the hydrolases from *Sphingomonas* sp. KT-1 and *Pedobacter* sp. KP-2, named Ss_hydrolase-1, Ss_hydrolase-2 and Ps_hydrolase-1, with tPAA and effects these enzymes have on tPAA biodegradation. Detailed investigations were performed to understand activities and elucidate synergistic action by using recombinant enzymes on tPAA. Finally, the individual and synergistic effect of the hydrolases have been investigated within an OECD 301F biodegradation test to showcase the potential of incorporating enzymes within existing standardized test to shorten testing times and better elucidate polymer biodegradation processes. These investigations aim to better understand the role of polymer molecular weight on microbial degradation and how this process is influenced by competent (extracellular) enzymes.

4.02.P-We335 Innovating on polymer degradation: Making two ends meet

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Polymers play a pivotal role in modern society by providing a multitude of technical functionalities in materials and products. They represent a vast and diverse aspect of the chemical space, often characterized by medium to very high molecular sizes and various functionalities. For polymers emitted down the drain, ultimate degradability becomes a favourable property. Yet, assessing the environmental fate of polymers poses scientific challenges, primarily due to their high molecular weight, which leads to low bioavailability impacting for example uptake in biodegradation studies.

In line with their emission routes, polymers used in Home and Personal Care products are evaluated for their biodegradation potential. However, the domain of applicability of standardized test guidelines, such as those from the OECD, does not include polymers. Therefore, the innovation of new materials faces two main challenges. The first is the need to accelerate the development of screening methods to inform product development, while the second is aligned to the need for the adaptation of standardized test methods to widen the applicability domain to polymers. In this presentation, we delve into methodologies for evaluating the biodegradation of polymers in aqueous systems during product development by use of screening and modified test methods.

At early stages of development, combining enzymatic assays with analytical techniques like ¹H NMR spectroscopy enables the characterization of changes in structural features. Additionally, candidate polymers are evaluated using standard or modified OECD biodegradation screenings studies. Adaptions including the extension of the test durations, are valuable approaches in comparing the biodegradation polymers with similar structural properties.

4.02.P-We336 Are Bio-Based Monomers Environmentally Safer Alternatives to Bisphenol A Diglycidyl Ether?

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In the last decades, bio-based epoxy resins have been gained relevance as more environmentally friendly alternatives to traditional petroleum-based epoxy resins, as the latter have proved to constitute serious hazards to the environment. A key challenge on the shift from petroleum-based to bio-based polymers is the replacement of one of the most widely used building monomers for epoxy resins, i.e., the diglycidyl ether of Bisphenol A (DGEBA), without affecting the final functionality of the resin. Among the bio-based alternatives to DGEBA, that have been developed and used, is the bisphenol-free diglycidyl ether of vanillyl alcohol (DGEVA SP s catalogue reference SP-9S-5-005 CAS 1584677-14-4) that could be obtained from lignin. In this context, the present work aimed at a comparative characterization of the toxicity of DGEBA and the bio-based monomer DGEVA to freshwater biota. Additionally, the toxicity of cystamine (SP s catalogue reference SP-2-4-001 - CAS 51-85-4), a bio-based hardener (derived from amino acids) also incorporated in epoxy resins, was also assessed. To achieve the established objectives,

the ecotoxicity of DGEBA and the two bio-based monomers was characterized by performing the following assays: 72-h growth rate inhibition with *Raphidocelis subcapitata*, 7-d growth rate inhibition with *Lemna minor*, 24-h acute toxicity assay with *Brachionus calyciflorus*, 48-h acute toxicity assay with *Daphnia magna*, and 96-h embryo acute toxicity assay with *Danio rerio*. Overall, DGEVA revealed a lower toxicity, comparatively to DGEBA, for the tested species, with EC50s always above 73 mg/L. Regarding cystamine, no mortality or malformations were observed in *D. rerio*. For the other tested species, the estimated EC50s of cystamine ranged from 6.18 to 63.84 mg/L. In conclusion, the obtained results confirm that DGEVA is a more environmentally safer alternative to DGEBA, thus, supporting the premise that it is a more sustainable bio-based building monomer of epoxy resins.

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4.03.P Statistics for Risk Assessment from Tried and Tested to New and Exciting Methods

4.03.P-Tu316 Applying Generalized Linear Mixed Models (GLMM) for the Evaluation of Ecotoxicological Endpoints in a Regulatory Context: A Framework Demonstrated by a Small Mammal Case Study

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Analysing data from ecotoxicological studies on small mammals to detect and evaluate effects of pesticide applications can be daunting due to the inherent variability of natural systems, the complex interactions between environmental factors, complexities such as non-linear dynamics, and the difficulties working with free ranging wild animals. Regression-based statistical approaches, including generalized linear and additive mixed effect models (GLMM, GAMM), have a long tradition in ecology to disentangle the influence of multiple factors on observed data. Such techniques have also gained attention in analysing data from higher tier enclosure and field studies conducted for environmental pesticide risk assessment. Nevertheless, GLMM analysis are often perceived as complex, leading to hesitation to account for their results in regulatory evaluations.

To enhance the uptake and understanding of GLMM and GAMM by regulators and ecotoxicologists, we aimed to present a structured framework of decision-steps to demystify the preparation, construction, validation, and evaluation of GLMM and GAMMs within the context of ecotoxicological field study data. This framework follows a two-stage approach where significance of treatment-related effects can be checked in both stages. A thorough data exploration, and model interpretation and visualization, complete the framework in a total of 7 steps.

The framework is demonstrated in a case study on voles exposed to a fungicide under field conditions. After exploratory data analysis, model alternatives were developed that are tailored to the different data types (counts, proportions, continuous data) recorded as endpoints of (semi-)field studies, and considering the repeated sampling within the same fields or enclosures and the potential non-linear dynamics of the endpoints over time. Different model alternatives were then compared through model selection, and the statistical power of the model evaluated through post-hoc Minimal Detectable Difference (MDD) testing. A final visualization for each endpoint assisted the interpretation of the statistical results.

The case study demonstrated the usefulness of the framework, to maximize the advantages of GLMM/GAMM analyses, namely, their adaptability to different data types, ability to incorporate all data within a single analysis (while excluding pseudoreplication), and ability to account for multiple influencing factors of direct and indirect interest to the study interpretation.

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4.03.P-Tu317 Four-Parameter Nonlinear Regression and Maximum Achievable Effect in Ecotoxicology: Just Visually Appealing or Biologically Relevant?

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In ecotoxicology, "EC_x" refers to the "concentration that causes x% effect" (OECD 2006). This seems to be a precise definition, but is typically only clear for EC_x values calculated for inhibitions of metric

variables from two- and three-parameter regressions, as they assume that the minimum of the affected variable is zero. In contrast, a four-parameter regression (4pr) assumes that the maximum achievable effect levels off at a value of the variable greater than zero. From this, several critical issues arise for ECx calculation: As the maximum effect does not necessarily mean 100% inhibition related to control, two types of ECx can be calculated. A relative EC50 is the concentration which shows the half maximal inhibition, whereas an absolute EC50 is the concentration which shows 50% inhibition related to control. The latter is what people intuitively associate with the meaning of ECx, whereas a relative ECx from a 4pr cannot be compared to ECx from other regressions. However, depending on the magnitude of the fitted maximum achievable effect, an absolute ECx from 4pr can be considerably higher than the corresponding relative ECx. Moreover, absolute ECx are limited to the maximum achievable effect, i.e., it may not be possible to determine an absolute EC50 at all.

In addition, a maximum achievable effect, and thus a justification for 4pr, may be claimed from a statistical point of view, even though it does not actually exist for the corresponding variable from a biological point of view. Why, for example, should a growth rate of a macrophyte not fall to zero if the exposure is further increased? Therefore, the choice of model should be based on the assumed underlying mode of action for a certain variable rather than on producing a visually appealing fit. This may be at the expense of the width of the confidence intervals of the ECx.

In summary, 4pr should only be used if there is a plausible biological mechanism to explain a maximum inhibition < 100%. In these cases, the absolute ECx values appear to more closely correspond with what is sought for regulatory purposes. In any case, if 4pr is applied, the calculation method used (relative or absolute toxicity estimates) must be clearly stated to avoid erroneous conclusions and to ensure transparency. Overall, the use of 4pr to determine ECx values for biological variables needs to be clarified in future guidance documents.

4.03.P-Tu318 Leveraging Bayesian Regression Models and Data Literacy for Better Decision-Making

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Environmental risk assessments (ERAs) rely heavily on the specific domain context and the availability of suitable data. Collecting high-quality data for ERAs can be challenging due to the variability in reliability across experimental sources and in silico information. The statistical analysis of information for ERAs is a current topic of debate, with an ongoing effort to revise OECD document 54 to improve the routine data analysis of ecotoxicological data.

For robust ERAs, understanding the data origin and its generation processes is vital. Challenges such as variations in experimental conditions, incomplete datasets, or measurement limits necessitate careful evaluation, especially in the context of ecotoxicology. These factors inform the selection of appropriate modelling strategies and, in the case of Bayesian data analysis, the implementation of meaningful priors.

Bayesian regression models (BRMs) provide flexible tools to address these challenges, allowing for the inclusion of diverse data types and the quantification of uncertainties. Beyond the Gaussian default, BRMs can incorporate alternative probability distributions tailored for count, survival, or concentration data. Features such as data weights (e.g., reliability), censoring (e.g., values below detection limits), and truncation (e.g., bounded response variables) can be modelled directly, reducing the need for a priori data manipulation. Advanced extensions, such as non-linear modelling (e.g., smoothing splines) and hierarchical components (e.g., random effects), further enhance BRM applications across all genres of ecotoxicology. The Bayesian approach allows for a direct probability quantification of a hypothesis, rather than the probability of the data alone.

Interpreting BRM results effectively is critical for assessing model quality and drawing meaningful conclusions for ERAs, such as through post hoc analyses and credible significance testing (e.g., ECX, BMD, or NSE). Technical aspects also include understanding, visualizing, and communicating posterior results and their uncertainties.

Collectively, these aspects can be viewed as data literacy for BRMs a critical skill for enhancing the quality of ERAs within regulatory frameworks. A stronger emphasis on data literacy, coupled with a broader adoption of BRMs through initiatives like the revision of OECD document 54, holds significant potential for improving ERA practices and fostering better understanding among stakeholders.

4.03.P-Tu319 Alternative Approaches to Deal with Difficult Cases in the Statistical Analysis of Ecotoxicological Data

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Endpoints such as Lowest Observed Effect Concentrations (LOECs) and Effect Concentrations (ECxs) derived from statistical analyses of ecotoxicological studies serve as one of the pillars for regulatory risk assessment. Sound experimental design and appropriate, fit-for-purpose analysis procedures are essential for well-informed regulatory decision-making. Decision flowcharts are valuable tools for guiding the analysis of ecotoxicological data and examples of decision flowchart can be found in OECD 54 and many OECD test guidelines. These flowcharts often require pretests to assess test assumptions such as normality and homogeneity of variance and to decide which test to use for certain type of data characteristics. Based on these pretests outcomes an appropriate test out of a pre-selected list should be selected. While decision flowcharts can efficiently guide routine analysis, they can occasionally yield misleading results. It is well-known that preliminary assessment in these flowcharts do affect the power characteristics of subsequent tests and can avoid the use of a low power test. However, it is not yet emphasized that the purpose of the flowchart is not to find the most powerful test for a dataset with a specific characteristic, but to find an appropriate method that is suitable for an endpoint with perceived general characteristics. In this presentation, real and synthetic data examples are presented to illustrate inconsistent power performance of different tests within the flowcharts, explaining when and why the decision chart does not work as expected for a given dataset and what can be done when the decision flowcharts fail. Our work highlights the importance of understanding the trade-offs between good experimental design, statistical power and robust analysis techniques in ecotoxicology. By emphasizing the need for appropriate test selection, the importance of proper usage and the potential pitfalls of decision flowcharts, we aim to contribute to improved experimental design and more reliable data generation and analysis for regulatory purposes. We propose using alternative robust approaches when deviation from the decision charts is necessary. Furthermore, we stress the necessity of conducting sanity checks, including visual inspections of model fit and data, following the analysis to ensure the validity of results. This holistic approach ultimately supports more informed regulatory decision-making in the field of ecotoxicology.

4.03.P-Tu320 Land-Use Suitability Decision Support System Tools: GIS & Geostatistical Models Versus CLEA & ICRL Approaches for Tangier Portsmouth Landfill Site Investigation

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Contamination in soils can create risks of harm to human health and the environment. This study reassessed the spatial distribution patterns of some selected heavy metals (Pb, As, Hg, and PAHs) on soils in the Tangier Road area of Portsmouth, UK using geostatistics approaches including GIS models against some previously used CLEA and ICRL approaches. The study analysed a total of 152 soil sample data-set obtained from the Department of Environmental Geology and Contamination (EGC), School of Earth and Environmental Science (SEES), University of Portsmouth, England, UK to determine whether contaminants are significant and their extent, nature, degree, and possibility of posing risk. This study, using data-set from the Parkman and Clayton report, reviews CLEA and ICRL approaches. ICRL threshold (now withdrawn) showed 500, 1, 10 and 50 mg/kg for Pb, Hg, As and PAH for allotment and corresponding values of 2000, 20, 40 and 1000 mg/kg for open spaces, parks and playing fields. The site was deemed to present risk based on the ICRL report. For allotment, the Soil Guideline Values (SGV) of Pb, Hg and As are 452, 26 and 43 mg/kg respectively, and the generic assessment criteria (GAC) for PAHs is 2.1. For residential, SGV for Pb, Hg and As are 450, 1.0 and 32 respectively. Using more environmentally friendly and scientific approaches, geostatistics and GIS models show non-normal distribution patterns of contaminants. While semi-variograms of Pb, Hg, As and PAHs indicate a blanket structure for As, and plume structures for Pb, Hg, and PAHs, GIS and Surfer kriging delineate varying degrees of hotspots. A threshold exceedance analysis of As provides evidence of low risk. The conceptual site model (CSM) suggests possible incomplete pollutant linkages. A comparative analysis of CLEA and geostatistics/GIS provides evidence that results from CLEA/ICRL models alone are insufficient to categorise the site as posing significant possibility of significant harm (SPOSH) across the entire landfill site. It is necessary to ascertain the source and origin of these contaminants and their bioaccessibility and bioavailability to determine the land's usability for future development.

4.03.P-Tu321 Workshop on Generating BMD10s for Wild Mammals Under EFSA's 2023 Bird and Mammals GD

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The EFSA GD 2023 on bird and mammal risk assessment for plant protection products has introduced new and challenging settings which require more technical guidance. Of these, the generation and use of BMD10 endpoints, instead of NOAELs, is probably the most notable.

The selection of endpoints from toxicology studies for BMD10 calculation, and the actual calculation, is not always straightforward. This is particularly evident for the area of wild mammals, where the BMD10s are based on the data set generated for the toxicological risk assessment, with typically 3 dose levels tested and reporting numerous measurements which may not always be relevant for wild mammals.

To address these challenges, a workshop has been organized with the objective to facilitate participation of the main stakeholders involved in the area of regulatory wild mammal risk assessment for pesticides in Europe under the EFSA GD 2023 (regulatory authorities, companies and CROs).

This workshop covers procedural and technical challenges, including data selection and processing, with the aim of contributing to the development of procedures that will aid in transitioning from chronic NOAELs to BMD10s when the EFSA GD 2023 will be implemented in the European Union later in 2025. This poster outlines the main discussion points, challenges identified, agreements achieved and proposed paths forward.

4.03.P-Tu322 Avian Reproduction Studies: Alternative Experimental Design and Statistical Methodology

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Recent EFSA (2023) guidance on risk assessment for birds and mammals proposes using regression or benchmark dose (BMD) modelling to estimate 10% effect concentrations in avian reproduction studies (OECD TG 206, OCSP 850.2300). Previously, studies aimed to determine a no effect concentration (NOEC) using primary 3 test concentrations plus a control, each with at least 16 male-female pairs. This study design, however, often lacks the sensitivity needed for accurate dose-response and BMDx estimation.

A collaborative working group of industry representatives and independent scientists collected a substantial avian reproduction study database. These data formed the basis for an extensive computer simulation study benchmarking several alternative experimental designs. The objectives were to (1) propose alternative experimental designs that support both BMD10 estimation and NOEC determination in avian reproduction studies while limiting bird use, preserving statistical power for detecting treatment effects, and maintaining the ability to meet the validity criteria in internationally-accepted test guidelines, most notably 29 eggs laid per hen in the control group; (2) refine statistical methods recommended by Green et al. (2022) to enhance effect estimation precision.

According to the simulations, two experimental designs would support both BMD10 and NOEC determination without increasing the number of birds used in the study, while still allowing all validity criteria to be met, provided that studies are allowed to continue until 29 egg are laid per hen: 1) 8 weeks of egg laying with 16 pens per group in 5 treatment groups and a control and 2) 8 weeks of egg laying with 18 control pens and 16 pens in each of 4 treatment groups.

The analysis concludes that some responses from some studies cannot be fit reliably by most commonly used dose-response regression models and a NOEC must be used. EFSA guidance also acknowledges this possibility.

The poster summarizes recommendations concerning model selection for BMD estimation and model averaging, along with acceptance and goodness-of-fit criteria. It also proposes solutions for challenging cases.

4.03.P-Tu323 Critical Evaluation and Meta-Analysis of Ecotoxicological Data on Per- and Polyfluoroalkyl Substances (PFAS) in Freshwater Species

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Despite the increasing concern regarding the ecological risks posed by per- and polyfluoroalkyl substances (PFAS), a lack of comprehensive understanding of their actual ecotoxicity remains. Through a meticulous examination of 91 peer-reviewed studies investigating effects at a population level and constructing probabilistic species sensitivity distributions (PSSDs), we present a state-of-the-science hazard assessment of PFAS in freshwater species. Using data subsets containing suboptimal data led to an overestimation of the predicted no-effect concentrations (PNECs) of PFAS. We report PNECs of perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonates (PFASs) in freshwater to be 4.8 2000 µg/L and 0.4 8.9 µg/L, respectively, derived from high-quality data. Statistical analyses revealed that both functional groups and carbon chain length significantly influenced ($p < 0.05$) the variations in toxicity observed among different PFAS. This study underscores the importance of obtaining high-quality PFAS ecotoxicity data to comprehend associated hazards. The PNECs of PFAS derived in this study are higher compared to those of micro/nanoplastics and persistent organic pollutants. Our research offers valuable insights into prioritizing the regulation of more toxic PFAS.

4.03.P-Tu324 How Normalization and Choice of Endpoint of Receptor Based In Vitro Assays Affect the Results

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Receptor based in vitro tests indirectly assesses to what degree a specific receptor interacts with a chemical. The strength by which it does so, however, depend on a range of factors such as e.g. cell viability and biochemical functionality, transfection stability, chemical potency, reader sensitivity etc. Both baseline and positive controls are therefore included in the assays. The purpose of the baseline controls is to establish a baseline signal reading of cells not affected by chemicals, while the purpose of the positive control(s) is to set a maximum response indicating the maximal signal the cells are able to perform when all receptors in the cells are occupied by a ligand with high affinity to the receptor, all the time (i.e. receptor saturation).

The aim of this study was to investigate the factors leading to variable baseline and positive controls, and evaluate the consequences of the methods used to normalize readings to baseline and positive controls for the final results of the assay. In addition, we wanted to assess the consequences of the models and endpoints used (linear versus symmetric and asymmetric sigmoid models, benchmark doses, low EC-values and 50% effect concentrations relative to positive controls).

We used data from three widely used receptor assays: the AREC32 cell line for Nrf2 activity, the DR-EcoScreen cell line for AhR activity and the VM7Luc4E2 cell line for ER activity tested on >300 wastewater effluent samples. The results showed that the normalization procedure had a large effect on the final results. First, we discovered that even ultra-pure evaporated solvents could have an effect in the AhR assay, visualising solvents as a confounding factor when working with up-concentrated environmental samples contained in a solvent. Subtracting or dividing by the baseline control, which are both recommended procedures depending on the assay and the protocol used, shifted the resultant EC-values fitted to normalized data. Variations in positive controls are also discussed in relation to the >100% induction ratios often observed. We conclude that raw data should always be given and normalization procedures should be explicitly described when reporting in vitro results, facilitating re-analysis of data.

4.03.P-Tu325 AI-aided Chronic Mixture Risk Assessment Along a Small European River in Central Germany

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The vast amount of registered chemicals leads to a high diversity of substances occurring in the environment and the creation of new substances outpaces chemical risk assessment as well as monitoring strategies. Hence, risk assessment strategies need to be modified to remain aligned with the rapid development and marketing of new substances. Here we performed a longitudinal chronic mixture risk assessment considering a real-world case study scenario with diverse anthropogenic impact types characterised by different land uses along a river in Central Germany. We selected six sampling sites with distinct land use types along the Holtemme River in Central Germany based on knowledge from prior studies. At each sampling site, we sampled river water using large-volume solid phase extraction. Following target chemical analysis using liquid chromatography coupled with high-resolution mass spectrometry, we were able to quantify 192 out of 457 substances above the respective limit of detection. For about 34% of them, we obtained empirical chronic effect data for freshwater organisms from the US EPA ECOTOXicology Knowledgebase. Furthermore, we applied the open-source artificial intelligence (AI) model TRIDENT to predict chronic toxicity for all substances. TRIDENT combines the use of a BERT-based transformer and a deep neural network to characterise the toxicity of molecular moieties from the SMILES code. A multi-scenario mixture risk assessment was conducted for three taxonomic groups, using the concentration-addition concept and considering various hazard and exposure scenarios. The results showed that the chronic risk estimates for all taxonomic groups were considerably higher when the empirical data was amended with data from in silico modelling. We identified hot spots of chemical pollution and our analysis indicated that fish were the most vulnerable taxonomic group, with pharmaceuticals being the most relevant risk drivers. Our study exemplifies the application of an AI model to predict chronic risk for aquatic organisms in combination with the consideration of multiple risk scenarios, that may complement future risk assessment strategies.

4.03.P-Tu326 Revisiting Some Decision Flowcharts for the Statistical Analysis of Ecotoxicological Data

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Decision flowcharts is an important tool in the analysis of ecotoxicological data and examples of decision flowcharts can be found in OECD 54 and many OECD test guidelines. In those decision flowcharts, often pretests need to be conducted for various test assumptions such as normality and homogeneity of variance to decide which test to use for certain type of data characteristics. For example, trend tests are prescribed when monotonicity test pass; nonparametric tests are used when inhomogeneous variances or non-normality are identified. Based on these pretests outcomes an appropriate test out of a pre-selected method list such as Williams or Dunnett, Bonferroni-Welch-t tests, stepdown Jonckheere-Terpstra test, Bonferroni U-test, Dunn's test should be selected.

While decision flowcharts are generally useful for guiding analysis, some of them contain redundant steps and they can occasionally yield misleading results. In this work, real and synthetic data examples are given to illustrate how and why a certain decision flowchart guided analysis can lead to results against intuition, what can be done when the decision flowcharts don't work properly due to pre-tests that lead to a test that is not appropriate for the concurrent data. For instance, a trend test goes wrong where a robust test can rescue. We advocate for the use of robust approaches instead of pre-tests in decision flowcharts. For example, Delacre et al. (2017) have proposed that psychologists should by default use Welch's t-test instead of Student's t-test or the two-step procedure (first performing Levene's test, then deciding which test to use), despite the latter's marginally better power when equal variance assumption is true. Similarly, we suggest adopting the more robust Dunnett test with unequal variance assumptions by default rather than the current practice of using standard Dunnett test which requires equal variance for valid results. We question whether it is worthwhile to choose different tests in case one is a few percent more powerful in specific data conditions through simulation study. Additionally, we discuss other robust approaches, such as using sandwich variance estimators for multiple contrast test of Dunnett and Williams (Jaki, 2013) that is robust against variance heterogeneity in balanced and unbalanced designs. Finally, we propose that a sanity check including visual inspection of the model fit and the data should be always conducted after the analysis.

4.03.P-Tu327 Equivalence Testing in Honeybee Semi-Field Studies

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In 2023, the European Food Safety Authority (EFSA) released the Revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). This revision introduced numerous significant modifications to the environmental risk assessment (ERA) of pollinators, including fundamental changes to the statistical evaluation of honeybee (*A. mellifera*) semi-field and field tests shifting from standard null hypothesis significance tests to equivalence testing. Unlike conventional null hypothesis significance tests which are designed for demonstrating the presence of effects, equivalence tests aim at demonstrating that effects fall below a pre-specified threshold. While this approach is successfully used in the field of pharmacology, its applicability to honeybee (semi-)field data within the context of ERA remains uncertain, especially considering the higher intrinsic variability associated with these test systems and the specific protection goal set at 10%. In the present study, we use negative control data of Bayer AG semi-field honeybee tests to quantify the test system's inherent variability for the main endpoint of colony strength (i.e. number of bees) at the end of the exposure period. Based on the assessed variability, we conduct data simulations to explore which true effect sizes can be reliably (i.e. with high enough (80%) power) detected as being below the 10% threshold within the equivalence testing framework. We discuss the implications of these results for the design of honeybee semi-field studies and for the proposed pollinator ERA.

4.04 Mechanistic Effect Models and Statistical Methods in Regulatory Science: Progress and Innovation for Environmental Risk Assessment?

4.04.T-01 Towards the Implementation of FAIR Principles for Mechanistic Effect Models (MEMs) in the Regulatory Environmental Risk Assessment (ERA) of Pesticides

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There is growing consensus that overreliance on experimental ecotoxicology and reductionist approaches in regulatory ERA may restrain risk assessment conclusions to a limited range of tested scenarios. Future risk assessment frameworks may require more explicit representations of ecological system complexity to better reflect case-specificity in risk assessment conclusions. In this context, modelling may become pivotal for designing new holistic risk assessments which might represent a paradigm-shift in prospective and retrospective ERA.

However, despite efforts aimed to define a role of MEMs in the regulatory ERA of pesticides, we remain some distance away from their routine implementation in prospective and retrospective ERA approaches. One noteworthy initiative undertaken by EFSA aimed to improve model acceptance in ERA has been the establishment of a Working Group (WG) on effect models in ERA, which aims to consolidate frameworks for the assessment of models in the prospective regulatory risk assessment of pesticides. In this presentation, we aim to present the views of the EFSA WG on how the careful stewardship of data, software, model documentation and model assessment documentation may contribute to overcoming some of the impediments behind the regulatory acceptance of MEMs in regulatory ERA.

Building on the original conceptualisation by Wilkinson et al. (2016), here we aim to present a regulatory perspective on the implementation of Findability, Accessibility, Interoperability and Reusability (FAIR) principles to MEMs in regulatory ERA.

Our work on the FAIR principles fits into and works in concert with other activities tailored to producing standards for the design, use and assessment of MEMs in ERA. These include establishing Good Modelling Practices, best practices for model documentation and model use or assessment.

In my presentation I will summarise the work undertaken by the EFSA WG on effect models on the conceptualisation of FAIR principles for effect models in the regulatory ERA of pesticides which will result in a publication by the first quarter of 2025. I will provide background on the community and regulatory

needs which motivated this activity. I will present the conceptualisation and implementation advice of the FAIR principles by the WG applied to MEMs in ERA, focussing on actions which may require prioritisation and long-term goals. Finally I will discuss challenges and blockers for their implementation.

4.04.T-02 BufferGUTS – A Toxicokinetic-Toxicodynamic (TKTD) Model for Above-Ground Terrestrial Invertebrates

Leonhard Urs Bürger and **Andreas Focks**, *Institute of Mathematics, Osnabrück University, Germany*
Mechanistic models can help to reduce animal testing by getting more information out of already existing datasets and can help to improve our understanding of effect processes. Toxicokinetic-toxicodynamic (TKTD) models link exposures and effects using mechanistic assumptions. The General Unified Threshold Model of Survival (GUTS) TKTD model is already deemed ready to use for aquatic species by the European Food Safety Authority (EFSA). To facilitate more event-based (feeding, contact, overspray) exposures in above-ground terrestrial systems, we developed a new generic TKTD model for above-ground invertebrates called BufferGUTS. It is based on GUTS but includes an intermediate buffer between the external exposure and inside of the organism. This buffer can be interpreted as residues on the exoskeleton or in the stomach, depending on the uptake route. Such an uptake behaviour is mechanistically reasonable and observable in laboratory experiments. Our model was tested against the reduced GUTS models and the honeybee specific BeeGUTS model (Baas et al., 2022) for 51 honeybee datasets (acute and chronic oral and acute contact) for 13 pesticides. BufferGUTS was able to perform (based on performance metrics) comparably or outperform the other models without using any species or substance-specific assumptions and the same number of parameters. The model performed as well for other non-bee arthropod datasets; thus we assume it can be used for all kinds of above-ground invertebrates. Having such a universal framework for above-ground invertebrates will enable the comparison and potentially even interpolation between species and substances, reducing the need for additional animal testing.

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4.04.T-03 Breaking Up is Hard to Do? Assessing Growth and Reproduction in *Lumbriculus variegatus* after Antidepressant Exposure by using a Novel DEB Model Approach

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Environmental risk assessment (ERA) of chemicals often relies on acute toxicity tests using high concentrations, focusing mainly on lethal effects. This substantially differs from the long-term, low-level exposures which can occur in the field and that may lead to sublethal effects over time. Dynamic Energy Budget (DEB) models provide a framework to bridge this gap by modelling energy allocation throughout an organism's lifecycle, allowing for more realistic exposure assessments. Although DEBtox models have recently gained attention, they are underutilized for sediment dwelling organisms like *Lumbriculus variegatus*, a species recommended for ERA. *L. variegatus* has an unusual reproduction by fragmentation, a dynamic currently not captured in conventional DEB models, which mainly describe sexual reproduction. This fragmentation can increase transfer of toxicants from mother to offspring. Here, we propose a mechanistic DEB approach to incorporate fragmentation, aiming to improve predictions of chemical impacts on model species with non-conventional reproduction types.

For the model calibration, we conducted a 28-day chronic experiment exposing *L. variegatus* to fluoxetine in sediment at three concentrations (0.0025, 0.25, and 25 mg/kg) and a control treatment. Growth, reproduction, and mortality were observed at seven distinct time points by distinguishing mature adults, mothers (recently split adult worms), and offspring. Our DEB model accounts for *L. variegatus* fragmentation by several extensions, including, among others, incorporating a juvenile starvation phase for head regeneration post-split. Results indicated two reproductive phases over the 28 days in the controls and a reproduction delay at higher exposure levels. By the end of the experiment, offspring numbers were significantly lower in the highest exposures, while growth of the offspring remained unaffected. This delay in reproduction highlights sublethal developmental effects often missed by conventional exposure assessment.

We conclude that monitoring the development of the organisms over time, rather than solely at the

experiment's end, gives important information about the chemical's mode of action. Furthermore, a better understanding of the organism's life cycle and the time point of reproductive events could improve standardized guidelines and reduce variability in chronic test results.

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4.04.T-04 Handling Experimental Variability in the Context of Calibration and Validation of DEB-TKTD Models: A Case Study using Cross-Laboratory Results for *Daphnia magna*

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Traditional environmental risk assessment of plant protection products (PPP) is usually based on controlled laboratory experiments. However, intra- and inter-laboratory variability in the toxicity endpoints are often observed for a same species-compound combination. This variability originates from various factors, including the genetic variability among organisms and strains, the organism health, the food quality or the test conditions in the laboratory. In practice, the exact origin of the observed variability is difficult to identify and the factors hard to disentangle from one another. In the context of mechanistic effect modelling, toxicity test data are used to calibrate and validate toxicokinetic-toxicodynamic (TKTD) models, such as DEB-TKTD models, which focus on sublethal effects at the organism level. These models have been identified as a potentially powerful tool for use in risk refinement as part the environmental risk assessment of PPP. So far, the impact of variability in the toxicity test outcomes on the calibration and validation process of DEB-TKTD models has not been clearly assessed. This is essential in case substantial variability is present among several data sets used in the model calibration or between the data set(s) used for calibration and those used for validation. In our case study, a DEB-TKTD model was calibrated and validated on two sets of bespoke cross-laboratory experiments conducted with *Daphnia magna* exposed to time-variable concentrations of the fungicide azoxystrobin. This study contributes to the portfolio of case studies of DEB-TKTD implementation for aquatic ERA, demonstrates how the modelling can overcome challenges of variability in biological data and identify the best modelling strategy.

4.04.T-05 Integrated Risk Assessment for Pyrethroids: A “Proof of Concept” Approach for Toxicokinetics’ Supported NAM-based Toxicodynamics

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Pyrethroids, widely used insecticides, pose potential risks to wild mammals due to their neurotoxicity and bioaccumulation in critical organs. Traditional risk assessment methods often fall short in capturing the full range of toxicological impacts. This study introduces an innovative approach that integrates toxicokinetics (TK) and mechanistic toxicodynamics (TD) to assess pyrethroid risks more comprehensively.

By combining in silico modeling and in vitro bioactivity data, this approach enables a detailed evaluation of aggregate and combined risks, focusing on internal dose-response relationships and organ-specific toxicity. A key innovation is the use of relative potencies to compare biological pathways and refine risk assessment methods, particularly for organs susceptible to long-term bioaccumulation.

This study provides a robust framework for ecological risk assessment, bridging the gap between experimental data and real-world exposure scenarios. The findings highlight the importance of considering intracellular concentrations in toxicological evaluations and demonstrate the value of combining TK and TD for a more accurate assessment of pyrethroid risks. This approach can be applied to other chemical classes, contributing to improved regulatory decisions and risk management strategies.

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4.04.P Mechanistic Effect Models and Statistical Methods in Regulatory Science: Progress and Innovation for Environmental Risk Assessment?

4.04.P-Tu328 Using GUTS Models to Predict Margins-of-Safety to Effects of Measured Pesticide Mixtures

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The extensive use of pesticides has raised environmental concerns due to their potential to contaminate soil and water and impact non-target organisms. Knowing that organisms in the environment are affected by a wide range of exposure to different chemicals, especially the effects of mixtures and their predictions, is crucial for Environmental Risk Assessment (ERA). Toxicokinetic-toxicodynamic models, such as the General Unified Threshold Model of Survival (GUTS) framework, can be used to predict mixture effects over time based on damage addition (DA) and independent action (IA). This study aims to predict the mixture effects of pesticide concentration measurements in a German monitoring study (KgM) using the GUTS model framework. The crustacean *Gammarus pulex* serves as a reference non-target organism. For the calculations of mixture toxicity, we categorized substances based on their modes of action (MoA) to identify, a priori, the compounds for which damage addition is anticipated. The exposure profiles obtained from the KgM were subsequently simplified by aggregating the concentrations of substances within the corresponding MoA groups, applying a weighting factor derived from the ratio of LC50 values. We aim to quantify the potential effects of the measured pesticide mixtures at each monitoring site by calculating LP50 values as a measure of the margin of safety relative to risk. Initial results show a wide range of LP50 values considering the two substances dimethoate and propiconazole and their corresponding MoA groups for the different measurement sites, indicating different pesticide contamination levels across the monitoring sites in Germany. Most of the LP50 values are quite high. Considering damage addition within the context of MoA groups, the toxicity analysis of both MoA groups, using dimethoate for neuromuscular substances and propiconazole for sterol inhibition, resulted in lower LP50 values, suggesting reduced margins of safety for non-target organisms. At some monitoring sites, LP50 values fell below 100 and even below 10, indicating potential acute risks not captured by single compound toxicity analyses. These results provide an initial overview but will be refined by including more substances and MoA groups. A long-term objective is to link these results to community-level risk indicators such as the SPEAR index.

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4.04.P-Tu329 TKTD Models for Aquatic Primary Producers – A New SETAC Working Group

Udo Hommen¹, **Annika Mangold-Döring²**, **Stefan Reichenberger³**, **Johannes Witt⁴** and **Cecilie Rendal⁵**, (1)*Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany*, (2)*Environmental Risk Assessment team, Wageningen University and Research (WUR), Wageningen, Netherlands*, (3)*knoell France SAS, France*, (4)*Bayer AG, Germany*, (5)*Syngenta, United Kingdom*

The use of toxicokinetic-toxicodynamic (TKTD) models to assess the effects of time-variable exposure of organisms to plant protection products is outlined in a Scientific Opinion of the EFSA PPR panel (2018). Since then, especially the GUTS model on lethal effects, is increasingly used. Some models are also available for assessing effects on the growth of algae or macrophytes, but a harmonized framework for calibration, validation and extrapolation of primary producer models does not exist yet.

A TKTD and growth model for the standard macrophyte test species *Lemna* sp. was developed by Schmitt et al. (2013) and subsequently refined and harmonized by the SETAC Lemna working group (Klein et al., 2022). The technical report is available on the SETAC website. As a follow-up activity, a new working group was established in 2024 with the aim to identify suitable workflows and measures for algae and macrophyte TKTD modelling in tier 2C (refined exposure experiments and modelling). In particular, the following objectives should be achieved in adherence to EFSA's Scientific Opinion on TKTD modelling (EFSA PPR panel, 2018) as much as possible but considering special properties of aquatic primary producers:

- 1) Development of a unified strategy for calibration and validation of TKTD models for aquatic primary producers
- 2) Development of a framework for generating moving time windows across FOCUS exposure series for use in Tier 2C assessments
- 3) Provision and ring testing of user-friendly open-source software solutions in the style of MOSAIC and

OpenGUTS

The working group has over 20 members and started to meet regularly since autumn 2024. The poster will present the current state of work and the working plan.

4.04.P-Tu330 A TKTD Module for BEEHAVEecotox – Combining BEEHAVEecotox and BeeGUTS *Dominik Lammers, Thomas G. Preuss and Vanessa Roeben, Bayer AG, Germany*

Honey bees play a vital role in providing the ecosystem service of pollination to both wild plant species and cultivated crops, making them a crucial consideration in the environmental risk assessment of plant protection products in the European Union. In this context, mechanistic modeling has emerged as a powerful tool for predicting bee exposure and effects in the field, gaining increasing acceptance within the regulatory framework.

The BEEHAVEecotox model represents a significant advancement as the first honey bee model to mechanistically link realistic field exposure with subsequent effects on various levels of bee colony. Utilizing standard regulatory study data, the model derives dose-response functions for effects resulting from contact and oral exposure, while also accounting for larval mortality.

Concurrently, the development of the BeeGUTS model offers a TKTD (toxicokinetic-toxicodynamic) model for interpreting and extrapolating honey bee survival data. This model provides a cohesive framework that integrates the effects from acute contact, acute oral, and chronic oral studies into a unified set of parameters, considering toxicity as a process over time. This innovative approach moves beyond single point estimates of toxicity and exposure to establish a holistic link between exposure and effect. Both models have undergone validation with independent datasets and have demonstrated strong predictive capabilities within their respective domains of applicability. Notably, the modular approach employed in the development of the BEEHAVEecotox model allows for the exchange or addition of modules as new knowledge becomes available.

In this presentation, we will unveil the initial results of integrating a new toxicity module into BEEHAVEecotox using BeeGUTS. The poster will shed light on the challenges and distinctions between the two approaches, while also comparing predicted results from semi-field studies.

4.04.P-Tu331 Applying a Toxicokinetic-Toxicodynamic Model to Risk Assessment of a Rooted Macrophyte, *Myriophyllum spicatum*

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There is growing interest in the use of modeling approaches to inform environmental risk assessment. Conventionally, lab-based environmental risk assessment studies test a gradient of concentrations to calculate dose response curves and inform risk to nontarget organisms. In assessing risk to aquatic plants, several model organisms are used, including *Lemna gibba*, a free-floating macrophyte, and *Myriophyllum spicatum*, a rooted macrophyte with high ecological relevance, where endpoints are based on effects on growth rate. However, lab-based studies are limited to specific environmental conditions and may not reflect the more complex conditions observed in the field. Additionally, traditional study designs may not adequately capture the relationship between molecules and their effects on an organism. Plant protection products aim to have low degradation half-lives and reduced environmental persistence, requiring lab-based studies to maintain artificially high and steady concentrations of a compound. In the field, exposure in aquatic environments is characterized by an initial pulse at the time of application and then a steady decline. Here, modeling allows for the extrapolation from lab-based conditions to more real-world scenarios, such as via Toxicokinetic-toxicodynamic (TKTD) models that describe species- and molecule-specific relationships.

We built on the TKTD model for *Lemna* and applied it to *Myriophyllum* and the interactions between its roots and the sediment. The model was calibrated and validated using laboratory and greenhouse studies, and outputs adequately reproduced observations. Using a rapidly degrading auxin herbicide as a case study, the model was applied to different chemical exposure scenarios reflective of expected environmental conditions. This application allows for a more holistic understanding of a chemical's potential influence on nontarget aquatic macrophytes and can provide additional information to apply to regulatory decision-making.

4.04.P-Tu332 Moving Towards Standardisation of Literature-Based Parametrisation of DEB Models for Regulatory Relevant Non-Target Arthropods: Insights from Four Case Studies

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In recent years, several regulatory guidance documents have endorsed the use of DEB-TKTD models (Dynamic-Energy-Budget based toxicokinetic-toxicodynamic models) to refine chronic pesticide risk assessment. These models allow both lethal and sublethal effects to be simulated as a function of constant or dynamic conditions of pesticide exposure and environmental factors like temperature. However, in order to calibrate such TKTD models to reproduce experimentally observed toxic effects adequately, at first the normal physiological behaviour of a species at unstressed control conditions must be explained well enough by the DEB part of the model. The Add-my-Pet repository provides many such models, although many regulatory relevant non-target arthropods (NTAs) are still missing. Furthermore, there is no prescribed procedure for how the data search for a new DEB model should be carried out and documented. In this study, we aimed to streamline this process by conducting a literature search for four terrestrial NTAs (*Typhlodromus pyri*, *Aphidius rhopalosiphii*, *Coccinella septempunctata*, and *Chrysoperla carnea*) according to the EFSA guidance on literature search, and provide documentation following the suggestions by the SETAC working group MAD. Subsequently, a DEB parametrisation was carried out for all four species, with particular attention being paid to the potential for standardisation on the one hand and the need for species-specific adaptations on the other. Three out of four species could be successfully parametrised in this way, while there were insufficient data for the last one (*T. pyri*). Overall, it was found that certain types of control data were replicated overabundantly in the literature, whereas data on the physical growth of the species were particularly scarce. Despite an attempt to use only existing model variants (towards standardisation), several modifications and model extensions were necessary to ensure the realism and usefulness of the models. These include a restructuring of typical life history processes in *C. septempunctata*, in which sexual maturation only begins after the pupa stage, as well as a new module for *A. rhopalosiphii* used to model its parasitisation rate as a function of reproductive investment, host type, host abundance, temperature, and timing during the reproductive phase. Both changes benefit from the modular nature of DEB models, and can be seen as extensions to the existing toolbox for DEB models.

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4.04.P-Tu333 Grassland as Surrogate Crop for Winter Cereals in Common Vole Field Effect Studies – Hypothesis Testing using Mechanistic Effect Modelling

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Mechanistic effect modelling can benefit environmental risk assessments (ERA) in various ways. Mostly it is used to evaluate risk in realistic scenarios or compare mitigation options. It can also be used to evaluate and improve field effect study set-ups by comparing different virtual study designs. Field effect studies are among the higher tier refinement options of pesticide ERAs for small herbivorous mammals focussing on common voles. In these ERAs the crop of interest is usually no primary vole habitat. Field studies in such crops yield usually only low individual numbers, which makes it difficult to detect potential effects. Thus, a common approach is to perform those studies in more favourable habitats for voles as grassland as surrogate crop. Here, we investigate whether vole field effect studies conducted in grassland are a feasible and protective approach.

We used the spatially explicit individual-based model for the common vole, eVole, to analyse population dynamics in a landscape consisting of both winter cereal and grassland habitat. We investigated the spatio-temporal population dynamics and the effects of pesticide applications in either of the two habitats.

Our modelling study exemplifies that throughout the year population density was higher and individuals were more homogeneously distributed in grassland compared to cereals, where voles more likely occurred in the field edges. Under pesticide application the vole population in treated grassland was affected at lower application rates and led generally to larger magnitudes and longer durations of potential effects compared to treated cereals.

The results underpin the assumption that field effect studies conducted in grassland are sufficiently protective to cover the risk assessment in secondary habitats like yearly field crops. First, in grassland, voles are present all year round at higher densities than in cereals, which allows capturing more voles to better detect population level effects. Second, the effects themselves are larger for treatments applied in grassland compared to cereals as voles are more frequently affected in grassland due to their higher densities and because of higher crop interception in cereals, particularly at later crop development stages.

Overall, this study underlines the potential of ecological modelling to support ERA not only directly providing additional evidence for the ERA process, but also for evaluating, planning and designing higher tier field options.

4.04.P-Tu334 APODEMUS, a Population Model for the Wood Mouse: Transparent Conceptual Model Development with Stakeholder Involvement

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The wood mouse, *Apodemus sylvaticus*, is the small mammalian omnivore focal species in the pesticide risk assessment scheme in Europe because the species occurs in agricultural landscapes across large parts of Europe and feeds on various diets. However, scenario-dependent individual exposure levels and potential population-level effects are challenging to assess in field exposure or effect studies. Simulation models can predict population-level effects from modelled life-cycle processes and individual-level exposure and effects. We present the concept for APODEMUS, A POpulation Dynamical spatially Explicit Model of the wood moUSE. Transparency is key in the devising of APODEMUS. Therefore, following best practices recommended in recent guidance documents by EFSA, we developed the conceptual model based on Pop-GUIDE. The Pop-GUIDE framework facilitates condensing all available knowledge and using it during the development of population models for risk assessment. For the conceptual model of APODEMUS, a comprehensive literature search and review provided the basis for the step-wise decisions leading to the conceptual model. In addition, these decisions were revised in a stakeholder workshop involving small mammal experts, risk assessors and regulators as well as ecological modellers. We will share insights from the conceptual model development and stakeholder involvement. This experience supported suggestions for better embedding Pop-GUIDE in the European risk assessment of small mammals. We demonstrate how a systematic development of a population model with continued stakeholder support can lead to a transparent tool for higher-tier risk assessments to improve risk assessment options and reliability.

4.04.P-Tu335 Individual-Based Models (IBMs) for Fish in the Regulatory Risk Assessment of Plant Protection Products - A Practical Method

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The current EFSA aquatic guidance document (2013) states that population models have great potential for use in the risk assessment of plant protection products (PPPs); however they have rarely been applied in a regulatory context. Specifically, in the risk assessment of PPPs, population models are included as a Tier III refinement option, i.e., to assess whether the effects observed at organism-level endpoints in standard OECD studies are expected to have a significant effect on populations under realistic exposure conditions. Population models do so by integrating aspects of life-history (e.g. reproductive rate), interactions between individuals (e.g. competition and predation) and population-level processes (e.g. density dependence) within a dynamic environment. In addition to predicting a pesticide risk, population models may also be used to improve our understanding of experimental data, identify focal (vulnerable) species, investigate the likelihood of population recovery and to assess natural variation in population abundance. This poster presents a case study to outline a stepwise approach for practically using Individual-Based Models (IBMs) for fish in a PPP risk assessment context, highlighting the critical decisions that need to be made regarding the modelling process. First, the species, model and scenario choice will be discussed. Then the implementation of exposure and effects and a simulation experiment will be presented, along with the assessment criteria for determining significant population level effects. Finally, the reporting recommended for a regulatory submission to facilitate an efficient evaluation will be described. Further work from the scientific community is still needed to better define models, focal species and scenarios for different uses in environmental risk assessments, as well as to better understand model outcomes. However, we believe there is currently a workable method for the use of population modelling in the risk assessment of PPPs by adapting off-the-shelf models.

4.04.P-Tu336 Determine Endpoints on Visual Effects for Terrestrial Plant (NTTP) Risk Assessment using Deterministic and Stochastic (Bayesian) Statistics

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For the authorization of plant protection products (PPP) in the EU the risk of negative impacts on non-target terrestrial plant (NTTP) is evaluated based on ER50 values for emergence, biomass, shoot length, and visually assessed phytotoxic effects observed in greenhouse studies (OECD 208 and OECD 227). Given the prerequisites for statistical evaluation of binomial distributed data from a dose/effect curve, the ER50 value can be derived for each of these effect parameters.

For visual phytotoxicity often scoring systems are used encompassing percentage ranges of the effects observed. One method (method 1) to deal with these ranges is firstly setting the value to the midpoint of its range and then using deterministic methods to derive the ER50 values (e.g., using R-package *drc*). For example, the midpoint 50% effect is used for the score 40-60% effect visually observed. Another method (method 2) is based on the Bayesian concept, where the effect range of the scores used is directly integrated in the statistical evaluation (e.g., R-package *rjags*). The authors compare the two approaches and in addition, with a third approach (method 3) where the concept of censored data (e.g. observed effects are > 30%) is integrated into the stochastic method (Bayes). The produced data sets were systematic aggregated to different score systems (ten, five, three) with equidistant and with unequal score levels, to understand the influence of the three modelling approaches on the effect value ER50 and the uncertainty (CI). The quantification of the midpoint model of the deterministic concept (method 1) and the Bayesian stochastic model using observation intervals (method 2) could give a better approximation than the Bayesian model with censored data (method 3), particularly of low score levels.

We want to share this comparison to facilitate the determination of robust endpoints for visual phytotoxicity from the studies used to evaluate the risk of PPP to NTTPs and to thereby support the evaluation of such data for PPP risk assessment.

4.04.P-Tu337 Tailored Test Designs to Address Actual Challenges Through Mechanistic Modeling – A Case Study for Temperature Dependent Effects of Imidacloprid

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In chemical risk assessment, extrapolations from laboratory tests to more realistic conditions are essential to address the effects of pesticides on individuals and populations under field conditions. In this context modelling approaches gained in relevance and acceptance to answer questions that cannot be addressed directly by experimental work. However, to further improve the response capability and thus the degree of reality these models can cover, it is important to design experimental studies that close common gaps in model parametrization and validation. This is best achieved by close cooperation of modelers, experimental ecotoxicologists, and ecologists.

Temperature dependence of effects is an example of an important factor which modulates the sensitivity of organisms to toxicants and must be considered to increase realism. Mechanistic effect models allow for the extrapolation and prediction of untested temperature scenarios (higher, lower, or variable) from models parameterized on standard test conditions.

Here, we present a case study with a series of tailored experiments for two insects, *Cloeon dipterum* and *Chironomus riparius*. A primary goal of this study was to obtain data on physiological temperature dependencies that could be applied to toxicokinetic toxicodynamic (TKTD) parameters in a General Unified Threshold model of Survival (GUTS) for lethal effects. Similar test designs were implemented for both species with a range of temperatures and exposure scenarios.

In a first step, effects at a standard test temperature (20° C) under chronic exposure were used to calibrate the GUTS model for both species separately. An Arrhenius function was added to the standard GUTS parameterization to predict temperature dependent rates for higher and lower temperatures. This model was able to predict effects that our experiments observed under the different temperatures and exposure conditions. The combination of mechanistic model and tailored experimental design proved that observed temperature dependency on physiological toxicity parameters and survival patterns can be predicted for a more realistic environmental risk assessment.

4.04.P-Tu338 How to Determine if an Effect in a Population Model Should be Considered “Adverse”?

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In recent years population models have been proposed as a potential tool for assessing the relevance of the population level effects for non-target organisms in a wide range of regulatory contexts, ranging from the ecotoxicological risk assessments of pesticides to the hazard assessment of endocrine disruptors. However,

the thresholds to define whether an effect is truly adverse are rarely detailed within the guidance documents. Traditional statistical methods for determining significant effects are not appropriate for population models as replication may be easily increased by performing more simulations and therefore change the value for statistical significance. In the academic literature, a priori default percentages of deviation from control simulations (e.g. 5-15%) have been used as the threshold for population response. However, this approach fails to consider the Normal Operating Range of the population resulting in a potentially different level of protection depending on the species being modelled. More recently, the updated EFSA Birds and Mammals guidance document (2023) proposed two assessment criteria for determining whether a population response is observed: (i) the mean of the exposed population falls below the lower 95th percentile of the control and (ii) the lower 95th percentile of the exposed population is consistently lower than the lower 95th percentile of the control. The EFSA criteria are promising because they avoid the use of arbitrary values set a priori by considering the actual dynamics of the population, and by using percentiles resist the influence of increasing model runs to change statistical thresholds. However, we found criterion 2 to be too strict and creating too many false positives. Comparisons of multiple sets of control simulations led to failure of this criterion and show of adverse effect where none should be present. Furthermore, we show that the strict interpretation of the assessment criteria meant that 100% of the scenarios exposed to a pesticide tested with different fish Individual-Based Models failed this second criterion (i.e. a population response observed in all cases). Further work is therefore required in order to establish a second criteria that is scientifically robust and consistent with the level of protection offered by criterion 1 before being used to assess population model outcomes. We will explore and present potential methods for determining whether a model outcome should be considered adverse.

4.04.P-Tu339 Spatial Heterogeneity of Environmental Exposure and Risk Assessment at Different Scales – Linking Exposure and Effects (ELINK-2)

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In 2007, the ELINK workshop convened experts in exposure and effect assessment to explore the impact of time-varying exposure patterns on aquatic pesticide risk assessments. Recently, the need for a new series of ELINK workshops was recognized and initiated to bring together specialists to advance the integration of exposure and effect modeling, and to develop environmental scenarios for risk assessment that consider spatial heterogeneity at various scales.

During the ELINK-2 workshop series, five working groups will discuss the implications of spatial heterogeneity at different scales:

- EL-LA - Aqua: Large scale landscape modeling related to aquatic non-target organisms.
- EL-LA - Terra: Large scale landscape modeling related to terrestrial non-target organisms.
- EL-ME: Medium/field scale modeling at the edge-of-field and field scale.
- EL-SA: Small scale modeling at the plant/soil interface scale.
- EL-GIS: Generic methodology of GIS data processing related to various scales.

The discussions will be based on case studies.

The most pressing issues for spatial modelling in European risk assessment identified in the first meetings of the 50-member working group are:

- Scenario development
- Geodata/Data availability
- Acceptability to authorities
- NTA risk assessment
- Test data sets/validation of models
- Complexity vs. pragmatism
- Ecological realism
- Ecologizing the exposure scenarios
- Variability/Uncertainty
- Quantifying the advantages of spatial modeling

4.04.P-Tu340 Advantages of Modeling Dose Response Mechanistically

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Environmental Risk Assessment (ERA) is based on measures or estimates of endpoints for exposure and hazard. Hazard assessment endpoints are based on statistical tests to estimate LOECs (Lowest Observed Effect Concentration) or dose response modelling for EC_x (Concentration for x% Effect) calculations. Most, if not all, studies generate more data than required for this endpoint calculation, so only a very small fraction of the generated data is used to estimate these endpoints, e.g., only the last day of a chronic 21-day chronic daphnia test. We compare conventional dose response methods to mechanistic TKTD (toxicokinetic-toxicodynamic) model approaches to derive the same endpoints. Examples demonstrate cases where mechanistic models provide deeper understanding of the ecotoxicological risk. We suggest that, where possible, collecting sufficient data from primary studies to capture the necessary endpoints for TKTD modeling can only improve understanding of and decisions on ERA outcomes.

4.05 Non-Target Arthropods: A New European Risk Assessment Guidance on the Rise

4.05.T-01 Insect Decline – Evaluation of Potential Drivers of a Complex Phenomenon

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Insect decline is a global environmental issue with far-reaching ecological consequences. Despite its significance, the causes of this phenomenon remain unclear due to the complexity of contributing factors and a lack of comprehensive historical data. This study aims to identify key drivers of insect biomass decline observed over 33 years in Western Germany by analyzing correlations between insect population trends and various potential environmental and anthropogenic factors such as changes in landscape structure and land management practices.

To address this challenge, we used long-term data from statistical records and market surveys, to track changes in landscape structure, land use, and agricultural practices. Key parameters included urbanization, grassland management, cropping practices, pesticide use, and climate factors.

Our results suggest that habitat loss and deterioration driven by land-use change and land management intensification are primary contributors to insect decline. Urban expansion significantly reduced insect habitats, while shifts in arable land towards bioenergy and feed crop cultivation, influenced by dairy farming intensification and renewable energy policies, exacerbated the trend. Notably, no direct links were found between insect decline and climate or pesticide use.

These findings suggest that balancing extensively and intensively managed areas, conserving natural habitats, and enhancing landscape structural diversity are critical to mitigating insect decline. Thus, the study offers insights into the drivers of insect decline and provides a foundation for targeted conservation strategies in Central Europe and beyond.

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4.05.T-02 Plant Protection Products and Ecosystem Services: Identifying Vulnerable Non-Target Arthropods and Linking Effects to Ecological Function

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The Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) is 22 years old and the European Commission has mandated the European Food Safety Authority (EFSA) to review the guidance for non-target arthropods (NTAs), in-soil organisms and non-target terrestrial plants. As part of this process the AENEAS project was initiated to advance the environmental risk assessment of NTAs by accounting for the impact of plant protection products (PPPs) on ecosystem services and ecological functions. Here we describe the implementation of a protocol to link PPPs effects on NTAs to potential impacts on key ecosystem services. A database of NTA species pivotal for pest control and pollination, sampled from six focal annual crops (wheat, barley, maize, oilseed rape, potato, beet) and three focal perennial crops (olive, apple, grape) across 37 EU members states was compiled. Representative NTA

groups contributing to pest control and pollination across crop type and across EU zones were identified. This analysis was performed separately for ground-dwelling and foliar pest control NTAs. Trait-based vulnerability analyses were used to identify vulnerable NTA within these representative families. The sensitivity of vulnerable representative NTA families to three insecticides was compared and chemical-specific vulnerability indices were calculated. Population modelling platforms (ALMaSS, Bumble-BEEHAVE) were used to assess the impact of PPP exposure on the population dynamics of vulnerable taxa within agricultural landscapes. The PPP effects on population abundance were translated to ecosystem functions using functional responses for pest control and by simulated flower visits for pollination. Landscape-level assessments considered subpopulation impacts (in crop, off crop) and landscape heterogeneity (crop diversity and proportion of natural habitat). Standard test species do not belong to the most vulnerable representative pest control or pollination NTA families and they may be less sensitive to insecticides than members of vulnerable representative NTA families for pest control and for pollination. Population modelling platforms show great potential for assessing the impact of PPP exposure on the population dynamics of vulnerable taxa within agricultural landscapes of different composition. However, limitations of both the population modelling platforms used currently limits the potential use of the protocol in regulatory risk assessment.

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4.05.T-03 A TKTD Data Interpretation of Non-Target Arthropod Tests

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Tests with non-target arthropods (NTA) are part of the requirements in the authorisation process for the registration of pesticides in Europe. These tests consist of exposing the predatory mite *Typhlodromus pyri* and the parasitic wasp *Aphidius rhopalosiphii* on treated surfaces, e.g., a glass plate in Tier 1 testing and treated leaves or plants in Tier 2 testing. This experimental setup should approximate the potential exposure of NTAs in the field.

The result of the tests is the lethal application rate that kills 50% of the organisms (the LR50), which is calculated from the dose-response curve from one particular observation timepoint of the test. We were interested in the suitability of these data for TKTD analysis and if this then could be used to integrate the glassplate test with the leaf tests, within one consistent framework. A total of 38 individual tests, comprising tests with individual insecticides, herbicides and fungicides, were available for this TKTD data interpretation.

In most cases the data consisted of both a glass plate test and a leaf test for both *T. pyri* and *A. rhopalosiphum*. In general, species appear to be less sensitive in the leaf tests than in the glassplate tests. This is likely caused by the altered exposure conditions in the leaf tests. The tests were carried out following the standard protocols, and it should be noted that these were not designed to carry out a data interpretation with a TKTD model. In most cases only 2 or 3 observations on effects in time were available, which is limited from a TKTD data interpretation perspective. Due to inconsistencies in the number of dead individuals over time, lack of observations on effects at intermediate points in time or inconsistent dose-response curves, some tests were not suitable for use in the TKTD approach. This left 26 tests (13 combinations of glass plate tests and leaf tests) available where the glass plate test could be used for an estimation of the decline of the concentration on the leaves.

The most important take-home message from this data evaluation is that when intermediate time-points with observations on effects are available and the result of the test is consistent, the standard tests can be interpreted with a TKTD model and that the result of the glass-plate test and the leaf test can indeed be integrated within one consistent framework.

4.05.T-04 Effects of Drift Exposure on Non-Target Arthropods: Insights from a Small-Scale Movement Model

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The European Food Safety Authority is revising its guidance on pesticide risk assessment for non-target arthropods (NTAs). This group encompasses a wide range of species with diverse behaviours and habitat

preferences, affecting their exposure potential. For NTAs in the off-field, usually the exposure via spray drift is considered as the main exposure route.

We developed a spatially explicit, individual-based model to simulate NTA movement in a given off-field structure with a spatial and temporal explicit exposure pattern. As an example, we use here exposure in a 2 x 2 m hedge area adjacent to treated crops. Pesticide residues, defined by three gradients, dissipate over time. NTAs are characterized by traits such as body size, movement speed, and preferred locations. In this example the model runs for 10 days with 10-minute time steps, simulating their movement as an uncorrelated random walk. NTAs pick up pesticides based on residue levels and contact area.

Simulations included four NTA trait groups: Aphidius-type (small parasitic wasps), Carabid-type (medium-sized ground beetles), Pisauridae-type (medium-sized spiders) and Typhlodromus-type (small predatory mites).

Residues were defined via three different gradients: "Field" side (highest contamination on the side facing the field), "Field and Top" (highest contamination in the top corner adjacent to the field and "Uniform" (even pesticide distribution).

Pesticide uptake varied significantly among trait groups and residue gradients. Due to higher body surface to volume ratios, smaller NTAs (Aphidius-type, Typhlodromus-type) accumulated more pesticides relative to body weight than larger NTAs (Pisauridae-type, Carabid-type). Behavioural preferences also influenced exposure: Pisauridae-type NTAs (randomly moving across the whole simulated area) showed no significant difference in exposure across gradients whereas Carabid-type NTAs (only located on the ground) were almost exclusively exposed in the Uniform gradient.

Overall, we show that NTA movement and behaviour along with residue distribution significantly influence exposure of the individuals in a population. Realistic risk assessments should consider these factors rather than assuming average exposure. This study shows again that mechanistic models can help us understand the complex interactions between behaviour and residue levels and improve our ability to predict risks of pesticide exposure to NTAs.

4.05.T-05 Developing a Framework for Identifying Sentinel Taxa for the Non-Target Arthropod Risk Assessment in Europe Using Field Effect Studies

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Non-target arthropod (NTA) field studies represent the highest tier of study available in the tiered regulatory process. Field studies have the highest degree of realism, but also a high degree of variability and unlike higher tier bee studies, NTA field studies are conducted with naturally occurring populations of multiple species. This results in large, complex datasets involving hundreds of different taxa with differing compositions and abundances, as a result these studies can be difficult to interpret and ascertain what is a potential effect of a plant protection product (PPP) on populations and communities versus natural background and seasonal variability.

In addition, the current NTA risk assessment in Europe (ESCORT 2) and Terrestrial Guidance Document (SANCO/10329/2002 rev 2 final) are currently undergoing revision meaning there are discussions ongoing in terms of what to protect, where to protect it, over what time period and with what degree of certainty (Specific Protection Goals SPGs).

The compiled NTA field effect database and its analysis represent a powerful resource for the development of the future risk assessment scheme for NTAs. In the first instance, it allows to derive recommendations for future field trial design and for its data analysis and interpretation. Further, the identification of sentinel taxa is not only relevant for the evaluation of field effect studies but also for the development of ecological models. Considering the spatial and temporal scale of NTA field studies and the number of taxa that are observed, ecological models are expected to gain in importance in the future NTA higher tier risk assessment in Europe. As it will not be feasible to develop models for all taxa potentially present in an area, a framework to understand the key representative taxa is therefore essential for model development. In addition, the database is valuable for analysing the spatial and temporal variability of taxa or ecological traits, thus making it a valuable resource for establishment of Normal Operating Ranges or/and Specific Protection Goals.

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4.05.P Non-Target Arthropods: A New European Risk Assessment Guidance on the Rise

4.05.P-Mo315 The Effect of Targeted Vegetation Sampling on Diversity and Variation of Mite Populations

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In ecological risk assessments (ERA) for plant protection products (PPP) mites are a key focus group, particularly in higher tier field studies involving non-target arthropods (NTA). Their composition (diversity and abundance) in the field is influenced by various abiotic and biotic factors, such as temperature, food availability, presence of predators or plant species composition (especially for herbivorous mites). As a result, high spatial variation is regularly observed, posing challenges for data analysis. In higher tier field studies in off-crop meadow habitats, mites are typically collected from vegetation samples, which involves indiscriminate sampling of all plant species (and the mites residing on them) from a random location of a defined size in the plot.

Here, we investigate whether targeted sampling (focusing on mites from target plant species with particular characteristics) can produce less variable data sets compared to the conventional indiscriminate approach (described above). Additionally, we assess how representative the mite community obtained through targeted sampling is in comparison to that obtained through indiscriminate sampling.

To explore the influence of plant type on mite composition in meadow habitats, we examined the effects of plant characteristics on mite diversity and abundance. We compared the mite composition on monocot plants versus dicot plants, and on hairy versus glabrous plants. Our focus was not only on differences in mite community composition between these plant groups, but also on the role of plant characteristics in reducing spatial variation in mite composition.

Our findings revealed no significant difference in mite diversity between monocot and dicot plants. However, significantly higher numbers of mites were found on hairy plants compared to glabrous plants. Moreover, mite abundance data from hairy plants exhibited less spatial variation between plots than data from glabrous plants.

These results suggest that targeted mite sampling could be a promising approach for higher-tier NTA field studies. It may (1) yield a comprehensive mite data set comparable to that obtained through indiscriminate sampling while (2) exhibiting reduced spatial variation, thereby providing a more robust basis for statistical analysis of PPP effects on mite populations and communities.

4.05.P-Mo316 Non-Target Arthropods: Effect of Sampling Effort on the Data Quality in Higher-Tier Field Studies

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In higher tier field studies conducted to assess the effect of plant protection products (PPP) on non-target arthropods (NTA), the composition of the arthropod s community is influenced by several biotic and abiotic factors (e.g., food availability, predator-prey interactions, etc.) on different spatial and temporal scales. For a good risk assessment, it is necessary to sample a representative proportion of the NTA community in terms of diversity and abundance, the latter having a big impact on the quality of the data analysis. However, there is a trade-off concerning the sampling effort: While data quality increases with increasing sampling effort, the feasibility of a study (field size, workload, costs) decreases.

Usually, in NTA field studies, four replicates per treatment are used. Here, we investigate how sampling effort affects (1) the composition of NTA (diversity and abundance) and (2) the number of taxa in a data set. For this study, we compare data sets of higher-tier NTA field studies with replicate numbers ranging from 3-5 (for off-crop) and from 2-4 for apple full fauna studies. We (3) relate the identity of those taxa, which were abundant enough to be included in population level analyses, to the list of taxa, which are supposed to be included in such studies according to De Jong et al. (2010). Special focus is put on the effect of the replicate number on the variability, and thus robustness, of the data sets.

4.05.P-Mo317 Non-Target Arthropods: Differences in Sensitivity to Pesticide Treatment In-Field vs. Off-Crop?

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Higher tier NTA studies measure the responses of local communities to the exposure of a plant protection product in highly complex natural systems in contrast to lower tier lab studies, where experiments are conducted under predefined conditions. These natural systems are complex, and NTA communities are shaped by biotic and abiotic factors, which increase the variability in the system. Consequently, several factors could influence the response of the arthropods to the pesticide exposure. Habitat structure and condition (e.g., vegetation cover), abiotic factors (e.g., temperature, humidity), exposure routes and the

biology of the arthropods (e.g., habitat preference, feeding strategy) could affect such differences in response.

In our experiments, we see species belonging to certain families of spiders (Araneae) and beetles (Coleoptera) responding differently to the treatment with plant protection products, which cannot be explained by the arthropods physiology, only. The phenomenon could be caused by long term exposure of different types of pesticides or by the differences in structure, abundance, and diversity of arthropod communities from different habitats.

Here, we investigate if the differences observed in the effect of the application of plant protection products on closely related taxa is correlated with abiotic factors (e.g., temperature, humidity) or habitat structure (off-crop vs in-field).

4.05.P-Mo318 Proposing Data Quality Criteria for Environmental Stressor Identification on Insect Biodiversity

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The German Environment Agency (Umweltbundesamt UBA), in collaboration with academic partners, conducted a monitoring program on small agricultural streams across Germany. The final campaign report concluded that the detected concentrations of pesticide active substances are the dominant stressor for vulnerable insects and that the current regulatory framework for pesticides in Germany is not sufficiently protective for aquatic macroinvertebrate communities. The authors attributed the potential decline of insect biodiversity to pesticide pressure based on a multiple linear regression analysis. We critically reviewed these conclusions by analyzing the methods applied using the raw data that are accessible on Pangaea. Our investigation into data consistency and methodologies revealed discrepancies that challenge the original findings. Specifically, we found inconsistencies and a lack of transparency in data processing and gap-filling practices. Moreover, discrepancies in the temporal alignment of data collection across different parameters suggest potential flaws in the multiple linear regression analysis. We further identified methodological issues in the determination and interpretation of the pesticide pressure index T_{Umax}. Additionally, our critique questions the reliance on single-value biodiversity indices like SPEARpesticides over the nuanced insights that multivariate analysis offers. These shortcomings undermine the reported effects of pesticide stressors on macroinvertebrate communities. We will propose data quality criteria that will support securing an appropriate level of data consistency in environmental data for the purpose of identifying primary environmental stressors on biodiversity

4.05.P-Mo319 Establishing Normal Operating Ranges of Key Non-Target Arthropod Taxa: Comparison of Approaches and Taxonomic Resolution

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A normal operating range (NOR) describes variability in a system property, for instance, it can represent variability in individual properties such as body sizes, or variability in a population property such as in abundance or biomass. The latter is of specific interest for pesticide risk assessment for Non-Target Arthropods (NTAs) because NORs are increasingly used to inform effect acceptability thresholds. However, there is currently no scientific literature or guidance document on appropriate approaches for determining NORs in a regulatory context of environmental risk assessment in Europe. However, data from industry field studies (generic and control replicates) and data extracted from peer-reviewed literature are a source of relevant information. Such datasets can support quantifying the magnitude of variability across relevant contexts (spatial, temporal) as well as sources of variability. This can then inform ecological (mechanistic effect) models that need to include appropriate sources of variability, so they are fit for purpose of NOR determinations.

In this study we look into a dataset consisting of a diverse group of species the non-target arthropods to identify appropriate approaches to analysing multi-species assemblage data in a way that can be used for operational NORs in a regulatory context of pesticide risk assessment. The dataset includes more than 90 generic and field effect studies conducted by industry. Both single species and higher taxonomic groupings are analysed to better compare variability in single species and in broader taxonomic groups. Here we present and discuss the conceptual approach to analyse and derive NORs from such

comprehensive, yet complex datasets, and illustrate preliminary findings in relation to their interpretation in a risk assessment context.

4.05.P-Mo320 The Impact of Selected Plant Protection Products on Two Coleoptera Species: *Aleochara bilineata* and *Poecilus cupreus*

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Despite the positive effects of pesticides in crop protection, there are significant risks associated with the detrimental impact of chemical substances on beneficial organisms that are naturally present in the environment. The aim of this project was to assess the impact of selected pesticides on two beneficial species of beetles: *Aleochara bilineata* and *Poecilus cupreus*. Both species were chosen as indicator organisms in the registration studies for chemical substances.

Since *A. bilineata* and *P. cupreus* have different lifestyles and life cycles, the studies are conducted in distinct ways, and various parameters are assessed to understand how these chemicals may affect them. The larvae of *A. bilineata* parasitize dipteran pupae to mature into adults; therefore, the studies focus on assessing the reproduction rates of these beetles and the potential mortality of adults when exposed to chemicals. In experiments involving *P. cupreus*, both adult mortality and their consumption of dipteran pupae are evaluated, as this species acts as a predator.

In the studies conducted as part of this project, three active substances commonly used in agricultural practices were applied: trinexapac-ethyl (a growth regulator), terbuthylazine (a herbicide) and deltamethrin (an insecticide). Simultaneously, in each test, the concurrent control group was prepared. Dimethoate was used as a reference material to verify the sensitivity of the organisms. The studies were conducted in the form of limit tests, which assessed a single concentration of each substance corresponding to field application rates.

No significant effects of trinexapac-ethyl and terbuthylazine were observed, both in terms of the reproductive output of *A. bilineata* compared to the control group and concerning the mortality and food consumption of *P. cupreus*. Deltamethrin showed varied toxicity to the tested organisms, depending on the field application rate. Dimethoate, as a toxic reference, confirmed appropriate sensitivity of both species of beetles to the chemical substances.

The results of the studies demonstrate selective responsiveness of organisms to different active substances and their concentrations, suggesting that various compounds can affect non-target organisms in species-specific ways.

4.05.P-Mo321 Moving Forward on NTA ERA: Contributions to Assessing the Inter-Species Sensitivity to Insecticides

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The assessment of the environmental safety of plant protection products (PPPs) in Europe includes the risk to terrestrial non-target arthropods (NTAs). As a data requirement in the first tier assessment, effect studies with two beneficial NTA species - the parasitic wasp *Aphidius rhopalosiphii* and the predatory mite *Typhlodromus pyri* - must be performed. Nonetheless, there has been a growing discussion if the current first-tier effect assessment sufficiently protects all vulnerable key driver NTA species in the field.

Additionally, the EFSA Panel on Plant Protection Products and their Residues defended that to reduce the uncertainty of the tier 1 risk prediction, test species should encompass taxonomic groups expected to show different sensitivity. A series of standard test protocols for different species of distinct trophic groups predators, pollinators and parasitoids have been published. However, due to the inconsistency in the testing methods developed, it is difficult to draw comparisons across species. One of the objectives of the AENEAS project (OC/EFSA/ED/2021/02) is to gain knowledge on the sensitivity of species representing the most vulnerable families of NTAs (key drivers for pest control and pollination) to PPPs. For that purpose, 16 NTA species were selected to be exposed to three commercially available formulations of insecticides with different modes of action - lambda-cyhalothrin, flupyradifurone and cyantraniliprole - via a single overspray application. Effects (mortality and abnormal behaviors) were assessed 4h after exposure and then each 24h until the end of the test, for a minimum of 48h. Medium Lethal Rates (LR50)

for 24 and 48h were derived, when possible, for each tested species per product, using the software PriProbit (Sakuma, 1998), and a graphical representation of those values was depicted via Species Sensitivity Distribution (SSD) curves, using the software ETX (RIVM, 2004). For the species already tested (9 out of 16), Karate Garden (a.i. lambda-cyhalothrin) was consistently the most toxic product, with exception of *A. rhopalosiphi* which presented a similar sensitivity to Mainspring (a.i. cyantraniliprole). For the three insecticide formulations, one of the least sensitive tested species was constantly *Chrysoperla carnea*. Furthermore, it is relevant to highlight that at least one of the species required in the first tier - *T. pyri* and *A. rhopalosiphi* - was one of the most sensitive species for the insecticides tested.

4.05.P-Mo322 How Do Landscape Compositions Influence Pesticide Effects in Landscape Models?

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Because pesticides are potentially harmful for organisms they were not intended to harm, environmental risk assessment (ERA) procedures were introduced to evaluate and mitigate that risk. These procedures span from tests on individuals to (semi-)fields experiments. Conducting risk assessment experiments on whole landscapes is not feasible for obvious reasons, but ensuring a healthy invertebrate population in the whole landscape is the ultimate goal of invertebrate risk assessment. Without healthy populations, some crucial ecosystem services (e.g. pollination or pest control) provided by such Non-Target Arthropods (NTAs) can no longer be sustained. To help identify potential blind spots in the current risk assessment, we analysed how different landscape compositions influence pesticide effects using a landscape model. We used the Animal Landscape and Man Simulation System (ALMaSS) from Topping et al. (2003) to model two predatory species, the spider *Erigone atra* and beetle *Bembidion lampros*, in four different European landscapes. Modelled agricultural landscapes were chosen to represent different levels of natural habitats and crop diversity. Pesticide application was modelled based on the properties of a real pesticide applied on one focal crop (oilseed rape) for 10 years. Effects on (sub-)population sizes and recovery times back to the control scenario were tracked. We found significant effects on sub-populations in non-crop areas already for permitted pesticide application rates. This shows action-at-a-distance effects of the pesticide in the simulation and thus a potential risk to populations not explicitly considered in ERA. For the four different landscapes we modelled, we could identify differences in effect strengths on species population sizes for the same pesticide amounts. Effect strengths appear to be influenced most by the proportion of the sprayed focal crop in the landscape and the proportion and composition (many small or few big patches) of natural habitats. While current landscape and species models can already provide new insights in landscape level effects, we see limitations that prevent the immediate use in regulatory risk assessment. To be able to use landscape models to support risk assessment decisions in the future, models need to be further developed and tailored to ERA needs.

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4.05.P-Mo323 Modelling the Accumulated External Pesticide Load of NTAs– Spatial and Trait-Specific Patterns for In-Field and Specific Off-Field Scenarios

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Recently, the revision of the Guidance Document on Terrestrial Ecotoxicology on plant protection products (i.e., non-target arthropods (NTA), non-target terrestrial plants (NTTP) and in-soil organisms) has been mandated to EFSA. For all organisms, the risk assessment consists of both the exposure and the effect assessment in several tiers. The Ecotoxicologically Relevant Exposure Quantity (EREQ) links an effect tier to an exposure tier. It is the exposure quantity that best correlates to an ecotoxicological effect. For NTAs, the current EREQ is basically the deposition rate on the surface of interest (plant canopy or soil). Based on current guidance the EREQ is calculated for the in-field and the off-field and is based on the application rate, the multiple application factor, and - for the off-field - the vegetation distribution factor and a drift factor. As a revised EREQ, the pesticide mass reaching an individual arthropod or the pesticide mass in and on the individual arthropod has been suggested. Additionally, at a spatial scale, a further distinction between the off-field, the off-crop in-field and the in-crop has been proposed. For the

revised EREQ, additional information about (i) the distribution of the applied product in an area as well as (ii) the movement patterns of a species plus body characteristics of that species need to be considered. A small-scale movement model for four theoretical NTA-species types, calculating the accumulated external pesticide load by individuals of a population in a defined exposure period, was developed recently by Becher et al. The distribution of the accumulated external pesticide load per individual differed significantly for model settings for the distribution pattern and the species-type defined by a particular trait combination. So far, the model by Becher et al. was run using assumed pesticide distribution patterns for some theoretical off-field only. In the present work additional pesticide distribution patterns as expected for the in-field have been established as well as specific off-field scenarios. The accumulated external pesticide load for the four NTA-species types used by Becher et al. have been determined and depend on the exposure pattern as well as their movement traits. The results provide valuable information on the identification of EREQs for all spatial scales for different NTA species trait types and demonstrate that it will be not simple to define a realistic worst-case.

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4.05.P-Mo324 Hoverfly Decline in the Context of Landscape Intensification

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Insect decline is influenced by various causal factors acting at different spatial and temporal scales and affecting different insect groups. Hoverflies are amongst the best studied insect taxa regarding behaviour, ecology, habitat and resource needs of adults and larvae. Hoverflies could therefore serve as surrogate group for many other insect taxa. The strongest decline trends occurred mainly in ubiquitous and widely distributed taxa not specialised to a low number of floral food sources. These species appear in high abundances if the habitat and resource needs are suitable. More specialised species with normally lower abundances are lower affected by the observed decline trend. From an ecological viewpoint this observation is remarkable, as specialised species with narrow habitat and resource needs are normally affected stronger by disturbances. The landscape analysis showed a possible explanation for this observation. From 1989 to 2014 especially the grasslands were converted to an intensified management. Extensive grassland habitats are an important habitat for many hoverfly species offering a wide variety of flowers as food source for adults. The intensification of the grassland habitats lowers down the ability of sufficiently high numbers of flower species subsequently dropping down the abundance of unspecialised hoverfly species. More specialised species can use the existing flower resources better resulting in weaker decline tendencies. There are no differences in the decline trends of resident and migratory species indicating that possible causes for the decline are also occurring within the investigated habitat. Further, the hoverfly community showed a clear dominance shift between 1989 and 2014. In 1989 the dominant species preferred open land/grassland and forest edge/shrubland habitats whereas in 2014 only species preferring forest edge/shrubland habitats were dominant. This shift also underlines that grassland intensification is a relevant factor for the hoverfly decline in this investigation site. For larvae the disappearance of pastures and extensive grasslands can be considered as important decline causes. Especially for species with rat tailed larvae highly dependent on cattle manure the decline trend was considerably high.

4.06 Bioremediation and Phytoremediation for Recovering Ecosystems from Legacy and Emerging Contaminants

4.06.T-01 Bacterial Pore Sealing as a Tool for Chemical Risk Reduction

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Immobilisation of organic pollutants is currently regarded as an effective strategy to mitigate chemical risks in soil by significantly reducing their bioavailability. In this study we explored the sealing potential of *Bacillus subtilis* DSM10, a bacterium well known for its biofilm formation ability and chemotactic response with biochar to reduce the aqueous diffusion of naphthalene. We also further investigated the possibility of enhancing immobilization by retarding chemical diffusion through micrometer-size pores clogged by bacterial biofilms. This was achieved using gamma-aminobutyric acid (GABA), a strong

chemoattractant. These combined approaches aimed to provide a robust method for limiting pollutant mobility in soil systems. To study the effect of biochar and microbial biomass on naphthalene diffusion without physical restrictions, biochar and *B. subtilis*, alone or in combination, were dispensed directly over ¹⁴C-naphthalene crystals inside conical glass tubes provided with a naphthalene trap filled with a 200 mM cyclodextrin solution. The second approach included the use of bioreactors. The lower reservoir was filled with a buffer containing ¹⁴C-naphthalene crystals and 10 mM of GABA. In the upper reservoir 1 ml of *B. subtilis* biomass or buffer without GABA was dispensed. Lower and upper reservoir were separated by a 3 µm pore size membrane. Also, there was a naphthalene trap filled with cyclodextrins attached to the upper reservoir. In all cases, the flow of naphthalene was determined by quantifying radioactivity in the cyclodextrin solutions. With the first approach, both biochar and *B. subtilis* significantly reduced diffusion- biochar alone by approximately 50% while *B. subtilis* alone by 87%, after 8 days. In the second set-up, the concentrated bacterial solution applied on top of the membrane decreased the flow of naphthalene through the membrane to less than 50% after 8 days. In this case the effect seemed to be enhanced by the chemoattraction exerted by GABA, resulting in the clogging of the pores. This work uniquely combines biochar and *B. subtilis* to immobilise naphthalene, highlighting the synergistic effects of bacterial biofilm formation and chemotaxis in reducing pollutant diffusion. The use of GABA as a chemoattractant to enhance bacterial activity and pore clogging offers a novel mechanism to limit contaminant mobility in soil and water systems.

4.06.T-02 From NatCom to Syncom. Optimisation of Hydrocarbon-Degrading Communities and Their Use in Contaminated Soils

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Bioremediation using bioaugmentation accelerates pollutant degradation by introducing microbial communities, yet natural consortia (NatCom) may include environmentally or medically undesirable strains. This study aimed to construct a synthetic microbial community (SynCom) from a NatCom isolated from hydrocarbon (TPH) contaminated soil in Noblejas, Spain. Manual and automated culturomics techniques using diesel as sole carbon source were carried out to isolate several candidate strains that were further characterized by 16S rDNA V3-V4 amplicon sequencing and whole genome sequencing, looking for their hydrocarbon-degrading potential. Thus, we formulated a SynCom composed of five strains taxonomically assigned as *Pseudomonas putida*, *Delftia acidovorans*, *Novosphingobium* sp., *Achromobacter aegrifaciens*, and *Rhodococcus jialingiae*. Additionally, *Rhodococcus* sp. WAY2, previously characterized for diesel degradation, was included to enhance metabolic pathways. SynCom stability was validated through successive culture passages in a minimal medium with diesel as the sole carbon source.

Field-scale experiments were performed using 20 tons of hydrocarbon polluted soils divided in two biopiles over a time span of 360 days. The first biopile did not receive any treatment and the second was biostimulated with vermicompost and bioaugmented with the SynCom culture. As expected, in the non-inoculated biopiles there was little change in diversity (determined as Bray Curtis distances) once the system was stabilised (after 90 days). On the other hand, in the treated biopile there were changes in the communities that were stable from day 240 after inoculation. These changes in microbial composition correlated with the decrease in TPH concentration. Thus, while in the control biopile a decrease of contaminants up to 58% of the original concentration was detected at 360 days, in the treated biopiles an average reduction of 63% was reached at 90 days, which was reduced to 29% at day 240, where it remained stable at around 24% at day 360. The main changes were observed primarily in the abundance of long-chain aliphatic hydrocarbons (>C₂₁-C₃₅) and polycyclic hydrocarbons with between 21 and 35 carbons.

Metagenomics and culturomics allow the construction of effective and safe SynComs to be used as inoculants in bioremediation technologies requiring bioaugmentation. We have constructed and tested one of these SynComs and proved its efficiency at field scale.

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4.06.T-03 Contaminated Soil Bioremediation by Indigenous Hydrocarbon-Degrading Bacteria

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Bioaugmentation has significant advantages for remediating soil contaminated with total petroleum hydrocarbons (TPH). This technique utilizes specific microbial populations, including biosurfactant-producing strains, to enhance the degradation of hydrophobic hydrocarbons by improving their solubility, mobility, and bioavailability. The aim of this work was to determine the effectiveness of a microbial formulation in soil bioremediation by evaluating the biodegradability of pollutants and the attenuation of toxic effects with an approach that includes chemical, microbiological, respirometric and ecotoxicological evaluation. Fifty bacterial strains from the ENEA-MIRRI Microbial Collection were screened for biosurfactant production using Oil Spreading and E24 assays. The best biosurfactant producers, such as *Pseudomonas glycinis* A2-5 and *Rhodococcus qingshengi* OSS19, were tested for compatibility and used to formulate a microbial consortium. Bioaugmentation tests were conducted in biometer flasks using a consortium of nine hydrocarbon-degrading indigenous bacteria at an inoculum of 10^6 CFU/ml. Experimental parameters were optimized by monitoring CO₂ evolution, TPH degradation, and ecotoxicity over 90 days. Chemical analyses via GC-MS revealed almost complete degradation of alkanes and alkenes and a 64-78% reduction in hopanes. The microbial load increased during the first 30 days before stabilizing, confirming the bioaugmentation process's efficiency. Ecotoxicological assays demonstrated a general reduction in soil toxicity over time. However, toxicity peaked at T15 in bioaugmented samples, particularly for *Chlorella vulgaris*, which remained highly sensitive throughout the experiment, possibly due to secondary metabolite formation. By T90, toxicity levels for *Daphnia magna* and plant species decreased significantly, indicating soil recovery. This study highlights the potential of bioaugmentation combined with biosurfactant-producing strains for remediating petroleum-contaminated soils. Future work will incorporate *Pseudomonas glycinis* A2-5 biosurfactant into the microbial formula to further enhance bioremediation efficiency. Ecotoxicological tests using multiple species proved effective in assessing soil health and recovery.

4.06.T-04 A Virtuous Cycle of Phytoremediation, Pyrolysis and Biochar Towards Safe PFAS Levels in Soil and Food

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Remediation of PFAS-impacted agricultural soil with destructive methods (soil washing, excavation, incineration, chemical oxidation) is challenging because of the diffuse character of the pollution. Furthermore, these destructive approaches will impair soil ecosystem services and cause carbon emissions. In-situ methods such as phytoremediation and sorbent amendment are less intrusive, more cost-effective, and align with the concept of nature-based solutions. Biochar from biomass combustion in the absence of air (pyrolysis), has been proposed as a sustainable sorbent material, due to its benefits in terms of carbon sequestration, nutrient recycling / waste handling, energy generation and contaminant immobilization. This is particularly the case if the biochar is made from contaminated biosolids, as pyrolysis can mineralize the PFAS. Pyrolyzing the contaminated biomass additionally alleviates the constraints of biomass disposal. Amendment with 1% sludge biochar or (activated) high-temperature (> 800 °C) wood biochar reduced PFOS leaching from contaminated soil by up to 92-99%, with notably better effectiveness for long-, than for short-chain (C4 and C5) PFAS (40-70%).

A high potential for terrestrial and aquatic plant bioaccumulation of PFAS has been shown for ultra-short chain and short-chain perfluoroalkyl acids (PFAA). Most studies were performed with crop species (e.g. corn, wheat, rapeseed) and rarely tested in field conditions. The main challenges for phytoremediation include not only high variabilities between the plant uptake potential of different PFAS (chain length, charge), but also high variations between plant species and field conditions, making it hard to predict removal efficiencies. With the suggested solution, we create a virtuous cycle when combining phytoremediation to accumulate short-chain PFAS in plant biomass, destroying of accumulated PFAS by pyrolytic treatment, and applying the resulting biochar to immobilize long-chain PFAS in agricultural topsoils.

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4.06.T-05 Bioremediation Strategies for Polychlorinated Biphenyl-Contaminated Marine Sediments: Advancing Methods for Sustainable Toxicity Reduction

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Polychlorinated biphenyls (PCBs) are persistent organic pollutants that pose significant environmental challenges due to their resistance to natural degradation, bioaccumulative nature, and potential toxicity to ecosystems and human health. Found extensively in marine sediments, especially near industrialized coastal areas, PCBs persist for decades, requiring sustainable remediation solutions. Traditional remediation techniques, such as dredging or chemical treatment, are costly, disruptive, and impractical for large-scale applications. Bioremediation as a sustainable alternative offers the potential to take advantage of microbial activity to degrade PCBs with minimal ecological impact. This study evaluated the bioremediation strategies, including bioaugmentation, biostimulation, and natural attenuation, to identify methods that effectively transform highly chlorinated and toxic PCBs into less harmful forms. The research employed microcosm experiments using sediments collected from highly contaminated sites. The bioaugmentation set mimicked the application of enriched microbial consortia, including species known for reductive dechlorination, to accelerate the breakdown of PCBs. A biostimulation set was operated to enhance the indigenous microbial activity through nutrient amendments, while a natural attenuation set was used to understand the sediment's natural ability to degrade PCBs. Microcosms were incubated under anaerobic conditions for six months, with samples collected at four-week intervals to monitor PCB degradation. GC-MS was used to measure changes in PCB concentrations and detect potential degradation products. Preliminary findings indicated that biostimulation led to sharp changes, including a significant reduction in higher chlorinated PCB homologs, while bioaugmentation exhibited slower but consistent decreases in higher chlorinated congeners and increases in lower chlorinated ones. Compared to the natural attenuation set, biostimulation showed faster changes, and bioaugmentation demonstrated steadier but sustained transformation over time. This study highlighted the potential of bioremediation strategies for PCB-contaminated marine sediments, contributing to the development of practical and sustainable remediation solutions.

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4.06.P Bioremediation and Phytoremediation for Recovering Ecosystems From Legacy and Emerging Contaminants

4.06.P-Mo326 Organic Amendments and Forest Restoration as a Nature Based Solution for Recovering a Construction Site Area with Spoil Material Treated with Lime

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Tunnel industry can cause habitat loss and degradation due to soil consumption and vegetation cover removal. Huge quantities of excavated material (Spoil Material, SM) are currently being produced for several large engineering and infrastructural project implementation. In line with circular economy objectives, SM can be used as a by-product if it meets specific EU guidelines (Waste Framework Dir. 2008/98/EC) and national legal requirements. Lime addition is often necessary to stabilize and move SM to its final destination site, in accordance with practical construction site procedures. However, lime-treatment strongly increases SM pH, making it unsuitable for filling areas designed for greening projects. This work reports a case study from a highway construction site, between Florence and Bologna (Bellosguardo, Italy), where a nature-based solution (NBS) was applied for decreasing the high basic pH of the SM and restoring the work site with a forest vegetation similar to the surrounding area. The greening project consisted in the use of organic amendments mixed to the SM (the bottom substrate), a 50 cm top layer of soil (surface soil previously removed from the construction area) and the planting of different endemic species (mixed oak wood). In order to test the effectiveness of the restoration project

considering the site-specific conditions, 27 mesocosms (15m²; 1.25 m depth), were setup. Three zero-km organic amendments (digestate; compost; de-oiled olive pomace) were applied. Two control conditions (only top soil, and no-treated SM) were also performed. The tree establishment, soil natural microbial community abundance and activity and soil pH and organic carbon were assessed at different depths (0-30 cm, top soil; 35-50 soil-SM intermediate layer, mixed with amendments; 50-60 SM) and times (12, 24 and 36 months). Adding amendments to SM (at 1 y), the pH values significantly decreased (from 12 to 10.7), thanks to the introduction of active microbial populations, organic C and N, if compared with controls. At 36 months, pH values (9) were close to those of the surface soil (pH 8). Total tree mortality was 15% and mostly affected oaks (*Quercus pubescens*, 38%). Tree performances among treatments did not show statistical differences even if one species (*Olea europaea*) grown in surface soil had the best performance. These results were useful for the real green restoration at the construction site, which is currently in progress.

4.06.P-Mo327 Compost Effectiveness in Improving Soil Quality and Plant Growth in an Agricultural Soil Contaminated by Antibiotics and Copper

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The use of organic amendments as fertilizers and copper as a fungicide in agricultural farming can be a source of emerging contaminants, such as antibiotics (ABs) and heavy metals. Consequently, agroecosystems can be an environmental reservoir for ABs and Cu, and their accumulation in plants and eventual transfer through food chains to animals and humans necessitate further investigations. The co-presence of ABs and Cu can adversely affect plants and their associated microbiome. To find solutions for improving soil quality and capacity to respond to contamination is a research priority.

The aim of the present work was to evaluate the effectiveness of a vegetable-derived compost (1%) in counteracting the negative effects of an antibiotic mixture (sulfamethoxazole, chlortetracycline and ciprofloxacin, 7 mg/kg each) and copper (CuSO₄ 30 mg/Kg) on the growth and development of lettuce plants (*Lactuca sativa*, var. Crispa). Moreover, soil and rhizosphere microbial communities were evaluated in terms of abundance (DAPI counts), structure (NGS) and enzymatic activities (dehydrogenase, phosphatase, α -glucosidase). Additionally, the possible presence of antibiotic resistance genes (ARGs) was also evaluated. Plant growth and physiology were assessed measuring biomass, number of leaves, leaf area, root elongation and chlorophyll content.

In compost presence, microbial abundance and activities increased, some microbial taxa were favoured (e.g., Gammaproteobacteria and Alphaproteobacteria) and others declined (e.g., Actinobacteria). In accordance with microbiological data, compost also promoted the highest plant biomass, leaf number and area, root elongation and chlorophyll content. Overall, the co-presence of copper and ABs increased the relative abundance of the *sul1* and *sul2* genes in soil, however, this phenomenon was significantly lower in plant presence.

4.06.P-Mo328 Plant-Assisted Bioremediation and Bioaugmentation Treatments for Recovering Multi-Contaminated Soils

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Inorganic pollutants and persistent organic pollutants (POPs) represent a considerable threat for environment and human health in both terrestrial and aquatic ecosystems. The green technologies, including Plant-Assisted Bioremediation (PABR) and Bioaugmentation, have the potential to be effective and sustainable tools for recovery and remediation of contaminated soils. This work reports a laboratory-scale case study, which was conducted for evaluating the efficacy of these technologies and design experimental protocols. The soil was collected from a historically contaminated area in southern Italy, exhibiting varying levels of polychlorinated biphenyls (PCBs) and heavy metals (HMs) (with average concentrations exceeding legal limits for urban areas for both organic and inorganic pollutants).

The microcosm experiments were conducted for 90 days in plant pots, considering four different treatments: (1) a negative control (only historically contaminated soil), (2) PABR with the *L. angustifolia* medicinal plant, previously demonstrated to thrive in HMs-contaminated soils, (3) bioaugmentation with a microbial consortium, comprising four hydrocarbon-oxidising bacterial strains belonging to the *Acinetobacter*, *Rhodococcus* and *Gordonia* genera, and (4) a combination of the microbial consortium with the plant species. The experiment was conducted at a temperature of 22°C, with a photoperiod of 8/16-night/day and a relative humidity of 80/65%-night/day.

At the start and the end (90d), analyses of soil properties (pH, EC), quantification of contaminants (PCBs and heavy metals), microbial abundance, dehydrogenase activity and 16S rDNA gene Next Generation Sequencing were performed.

The preliminary results suggest that the combined use of plants and microorganisms promoted microbial abundance and improved contaminant degradation at 90 days.

The analysis of microbial structure revealed that lavender and the bacterial consortium stimulated the proliferation of specific microbial populations such as Xanthobacteraceae, Geobacteriaceae, and Bacillaceae.

This study supports the effectiveness of microorganisms and plant interactions in increasing soil quality and promotes contaminant removal.

4.06.P-Mo329 Assessment of Microplastic Effects on Plant Assisted Bioremediation Strategy for Recovering Contaminated Soils

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Plant-assisted bioremediation (PABR) is a sustainable technology that exploits natural interactions between plant roots and rhizosphere microorganisms for degrading, immobilizing or extracting contaminants from contaminated soils. This approach is particularly effective in removing from soil a wide range of pollutants such as heavy metals, polycyclic aromatic hydrocarbons, pesticides, PCBs and dioxins. Recently, micro- (MPs) and nanoplastics (NPs) have emerged as new contaminants, posing major environmental challenges and serious concerns for soil biota health. MPs and NPs from intensive agronomic practices (e.g. mulching sheeting, organic fertilisers such as biosolids) and use of reclaimed water can have both a direct physical impact on soil structure and biota (e.g. microorganisms and plants) and act as carriers of hazardous chemicals. On the one hand, new experimental approaches are evaluating the use of plant technologies as a means of removing micro and nanoplastic from soil. On the other hand, few studies have evaluated the effects of microplastics on the efficiency of strategies such as PABR in multi-contaminated soils. Microplastics can alter soil physical and chemical parameters, as well as plant physiology and microbial diversity in rhizosphere. Alteration in soil pH can affect mobility and bioavailability of pollutants such as heavy metals and nutrients, and indirectly hinder plant growth and bioremediation processes. For example, root exudates, which have an important role for stimulating microbial communities in PABR, can be metabolically modulated and reprogrammed by presence of microplastics, affecting plant-microbiome synergic interactions. In this context, this work aims to review and discuss effects of microplastics on soil key abiotic and biotic factors (pH, electrical conductivity, organic carbon and soil nutrients, organic and inorganic pollutants, plant growth, microbial and fungal communities, radical exudates, soil amendments) which regulate PABR technology for recovering contaminated soils

4.06.P-Mo330 Collaborative Approaches to Water Management: Bridging the Global North and South

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Water scarcity and access to safe drinking water and sanitation remain critical global challenges. By 2040, it is projected that one in four children will live in regions of extreme water stress due to over-exploitation, population growth and climate change. The Global South is particularly vulnerable to these problems. To address the clean water crisis, a circular and inclusive approach to water resources management is essential. Expertise from European Union countries can address research needs and knowledge gaps in the Global South, while evaluating current challenges and best practices in the Global North.

The NEUTRAL4GS project brings together six academic institutions, one SME and one water utility to develop and test nature-inspired solutions for urban water management through co-creation and co-design strategies between partners from the Global South and the EU. The project focuses on different types of urban water, including stormwater, greywater and wastewater, and integrates a range of treatment technologies. The project aims to develop innovative engineering solutions and processes, including membranes, biomass carriers and bioremediation precursors, to improve water quality and meet specific challenging performance targets, such as the removal of micropollutants and heavy metals. Laboratory-scale test units of individual and/or combined technologies will be implemented to assess their feasibility. This presentation will focus on bioremediation approaches and technologies for wastewater treatment. Nature-based solutions (NBS) will be applied at the laboratory scale for controlled process studies focusing on priming microbial communities for enhanced micropollutants treatment. Microbiological interventions will be employed to enhance remediation processes, including the biodegradation of microplastics. This project task will collaborate closely with others to integrate optimized processes into NBS handling stormwater and greywater, where relevant.

Holistic sustainability and economic assessments will guide the development and prioritization of these technologies. NEUTRAL4GS aims to pioneer innovative water treatment solutions, ensure social acceptance and adoption of these technologies, and generate significant societal, environmental, and public health benefits. By raising awareness and involving local authorities and communities, NEUTRAL4GS strives to ensure the sustainability and long-term success of its outcomes beyond the project's lifetime.

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4.06.P-Mo331 Potential of Bivalves for Bioremediation of Wastewater – Comparative Assessment of Biofiltration and Biosorption

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Wastewater treatment plants are generally ineffective at removing some contaminants of emerging concern from wastewater, which represents a threat to the environment and human health. Several approaches have been developed to overcome this problem, bioremediation being a promising one. The bivalve *Corbicula fluminea* has advantageous attributes as a wastewater bioremediation agent considering its high tolerance and status as an invasive species outside its native range in Asia. Hence, this study aims to evaluate the potential of *C. fluminea*, considering both the living organisms (biofiltration) and the shells (biosorption), in the removal of some of the most frequent contaminants from urban wastewater, namely caffeine (CAF), carbamazepine (CBZ), diclofenac (DIC), fluoxetine (FXT), ibuprofen (IBU), metformin (MET), naproxen (NPX), paracetamol (PCT) and sulfamethoxazole (SMX), further appraising their ecotoxicological effects in the microalgae *Raphidocelis subcapitata*. Biofiltration efficiency was evaluated using 10 bivalves per 0.5 L during 48 h. Adsorption to milled shells was assessed under similar conditions (50 g shell/L). Posteriorly, adsorption was tested under fixed conditions: pretreated (pyrolyzed and acid-washed) shells, at higher dose (100 g shell/L), at pH 7.0. All tests started with contaminants at 0.5 mg/L. Biofiltration showed low efficiency, with removal percentages below 40%, except for FXT (93%) and PCT (59%). In contrast, milled shells removed mainly CAF (62%) and FXT (42%), with removal percentages below 40% for the remaining contaminants. Clams were more efficient in removing CBZ, DIC, MET, FXT and PCT whereas the opposite was observed for CAF and NPX. Biosorption by pretreated shells showed a removal efficiency higher than 50% only for DIC (80%), FXT (67%) and MET (53%). Toxicity of the FXT sample to the microalgae decreased after biofiltration but increased for most of the remaining samples. Unexpectedly, toxicity of CAF and FXT samples did not decrease after biosorption to milled shells, suggesting that toxicity cannot be explained based only on compounds removal. Overall, results suggest that living bivalves would not likely represent a viable solution for urban wastewater treatment. Biosorption using pretreated shells was successful and should be further explored in

adsorption tests with real wastewater to fully assess the potential of this approach for urban wastewater treatment.

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4.06.P-Mo332 Sorption of Road Runoff Pollutants to Wood-Derived Biochars

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Blue-green infrastructures, such as tree trench biofilters, have untapped potential to reduce pollutants in road runoff. Runoff is characterized by highly variable quantities and chemical loads that may overwhelm microbial transformation and lead to pollutant transfer to the subsurface. Augmenting biofilter systems with carbon-based sorbents may allow for fast transitory retention of pollutants in high load periods and continuous slow release for long-term biodegradation. Wood-based biochar thereby emerges as a sustainable and cost-effective sorbent. However, the role of physicochemical biochar properties in efficient transitory retention and slow pollutant release for subsequent biodegradation is still poorly studied. Here, we present the sorption equilibria and kinetics of four typical road runoff pollutants (naphthalene, methyl tert-butyl ether, 1,3-diphenylguanidine, and perfluorooctanoic acid) of untreated biochar (BC) and activated biochar (BAC) in batch experiments. Isotherm data for adsorption and desorption were well fitted by the Freundlich model. BAC exhibited a significantly higher adsorption performance, e.g. the equilibrium loading for 1,3-diphenylguanidine at an environmentally relevant concentration of 30 µg L⁻¹ in the aqueous phase is about 100-fold higher than that of BC. All model pollutants showed strong desorption hysteresis in both BC and BAC, with even greater hysteresis observed in BC. Sorption kinetics data were evaluated using the film-surface diffusion model to estimate surface diffusion coefficients for pollutants transport inside biochar particles. These parameters, together with Freundlich isotherm parameters, were applied to three different transport models to predict breakthrough curves under varying water flow rates and pollutant inflow concentrations. Based on the model results, suitable experimental conditions were selected to conduct column experiments. Experimental data were then used to validate the models and improve their accuracy to predict real-world scenarios. Our results indicated that BAC had a superior adsorption capacity and kinetics, and a lower desorption hysteresis compared to BC. The significance of predictions from batch tests combined with modelling tools for different scenarios in biochar-amended biofilters was discussed. These will form the basis for the assessment and control of microbe sorbent interactions for efficient bioavailability and biodegradation of runoff pollutants in tree trench biofilters.

4.06.P-Mo333 Industrial Hemp (*Cannabis sativa*) as a Dual-Purpose Solution for Soil Remediation and Bioenergy Production in Sewage Sludge-Amended Soils

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Sewage sludge (SS) reuse in agriculture is encouraged due to its nutrient-rich composition; however its high heavy metal content, potential environmental risks, and limited guidelines for safe application pose significant problems. Contaminants accumulating in SS-treated soils can harm soil organisms, reduce soil fertility, endanger food crop yields and enter the food chain, contaminate surrounding areas. Furthermore, excessive sludge application can result in long-term soil pollution, leading to the need to clean up amended sites. The use of energy crops in soils treated with SS is a sustainable dual-purpose method for soil remediation that combines it with bioenergy production.

The aim of this study was to assess the potential of energy crop industrial hemp (*Cannabis sativa* L.) for soil remediation and bioenergy production in soils amended with sewage sludge (SS) and sewage sludge char (SSCh) at application rates of 25 200 Mg/ha. SS and SSCh application highly affected Cr, Cu, Ni and Zn concentrations in soil (ANOVA, FSS > 21.80, FSSCh = 35.20, p < 0.001) and led to increased metal concentrations (p < 0.05). Soil amended with 25-100 Mg/ha SS and 25 Mg/ha SSCh had moderate heavy metal contamination. Application of 200 Mg/ha SS and 50-200 Mg/ha SSCh caused significant pollution. Our findings show that the application of SSCh resulted in more polymetallic soil contamination than the soil amended with the same SS dose. Heavy metal concentrations in soil were significantly reduced after industrial hemp cultivation, with higher removal efficiencies at moderate sludge application rates (25 50

Mg/ha). The heavy metals removal efficiency could be ranked $Zn > Cu > Cr > Ni$. Only marginal removal was detected for Ba, Fe, Na, Ti and Al. These reductions underscore industrial hemp's effectiveness in mitigating heavy metal posed environmental risks.

The optimal fertilization with SS or SSCh could be up to 25 Mg/ha, when the highest efficiency of contaminant removal from the soil and the highest plant biomass production and bioenergy production were observed. Higher application rates increased the risk of heavy metal contamination, emphasizing the importance of optimized sludge usage. The study highlights the dual benefits of industrial hemp cultivation in mitigating soil pollution and contributing to renewable energy sources, offering a sustainable strategy for managing sewage sludge and its environmental impacts.

4.06.P-Mo334 How Persistent are Persistent Organic Pollutants in Soil? Exploring Bioavailability
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Nature-based solutions (NBS), such as bioremediation and phytoremediation, offer promising avenues for soil restoration. Traditional NBS approaches focused solely on total pollutant removals have proven unpredictable and often fall short of legislative remediation goals. This is particularly true with persistent organic pollutants (POPs) such as PAHs. With a focus on bioremediation, this overview presentation will explore the paradigm shift towards addressing bioavailability of POPs, surpassing mere total pollutant removals, to reach acceptable risk reductions. To achieve this, two questions must be addressed: (1) How to influence bioavailability processes related to the biodegradation of PAHs? and (2) How can this knowledge be used to sustainably reduce chemical risks in soil? In relation to the first question, considering that contaminants that are not bioavailable to degrading microorganisms can be bioavailable to humans and ecological targets, it is necessary to bring forward microbial activity to acceptable levels of risk reductions. Our nature-based engineering components were demonstrated to enhance biodegradation and reduce environmental risks, mainly channelled through the water phase, at various levels of bioavailability processes, encompassing interactions between contaminants and soil, their transportation, and biological processing. The specific focus in our research was to unveil low-risk strategies such as the controlled increase in bioavailability to microorganisms targeting slow-desorption pollutant fractions with (bio)surfactants, fine-tuning the deposition and motility of microbial degraders, co-mobilizing non-motile inoculants, and effectively capturing pollutants through plant/biochar arrangements, showcasing their potential to transform soil remediation practices. In relation to the second question, operating on bioavailability requires the use of standardized methods that can systematically be used in bioremediation to measure bioavailable fractions, evaluating more realistically operations and end-points, in addition to assessments based on total concentrations only. Desorption extraction (Tenax) is especially useful in bioremediation and constitutes the basis for an ISO standard on soil quality. Our group has already used this approach in the evaluation of bioavailability-oriented bioremediation, proposing application paths in the context of sustainable recovery of soils affected by military activity.

4.06.P-Mo335 Influence of a Natural Nonionic Surfactant on Biodegradation of Slowly Desorbing PAHs in Contaminated Soil

Rosa Posada, Alicia Fernandez-Vazquez and Jose Julio Ortega-Calvo, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Spain

In many cases, the residual pollutant concentrations after bioremediation suppose unacceptable risks, especially for hydrophobic organic compounds such as PAHs. The half-lives of these compounds are very high if are present as slowly desorbing forms, and may remain in the contaminated soils after bioremediation, still causing potential toxicity. The design of strategies to enhance the biodegradation of the slowly desorbing compounds is therefore needed in the bioremediation field. The use of biosurfactant agents, such as rhamnolipid, have been suggested as the environmentally friendly technology for the remediation of soils contaminated with hydrophobic organic compound. Plant-produced surfactants, such as *Quillaja saponin*, constitute a realistic alternative to microbial surfactants, as related to costs. Our objectives were: to characterize the initial desorption kinetics of native PAHs present in this contaminated soil to determine the extent of slowly desorbing fractions, to use ¹⁴C-PAHs radiorespirometry determinations as a physiological indicator of biodegradation, to study the effect of the saponin on the biodegradation and bioavailability of these compounds and to propose mechanisms to improve the action of this biosurfactant on slowly desorbing PAHs in contaminated soils. The soil used was a heavily

creosote polluted soil with a PAHs total concentration of 4075 mg/kg. Saponin, a non-ionic biosurfactant, was added at concentration of 7 mg/g (w/w). The desorption kinetics, which was determined by Tenax extraction, was a necessary tool in the optimization of saponin role in bioremediation of PAHs. Saponin was added at 56 days, when the remaining PAHs were present in a slowly desorbing form as a result of the biodegradation of fast desorbing PAHs by the native microbial population of the soil. The biodegradation percentages of total PAHs (sum of 16 EPA PAHs) achieved were 59 % and 90 % for the natural attenuation and bioestimulation-saponin treatments, respectively. Thus, the data obtained by adjusting the desorption kinetics successfully predicted the right time for biosurfactant application during bioremediation. The results obtained show that an appropriate integration of the natural surfactant into bioremediation of PAHs contaminated soils, promoting biodegradation of the slow desorption fractions, improve the bioremediation performance and have lower costs and environmental impacts.

4.06.P-Mo336 Improving Remediation of Military-Contaminated Soil by Natural-Based Solutions

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Bioremediation is a main natural-based solution for the clean-up of polluted soils. Pollutant biodegradation is improved by stimulating the activity of the autochthonous microorganisms through the improvement of soil conditions (biostimulation) and/or through the addition of allochthonous microorganisms with specific metabolic traits (bioaugmentation). In heterogeneously polluted soils with organic xenobiotics, it is essential to guarantee the dispersal of the inoculants over the volume of soil and to promote the bioavailability of the pollutants, which will in turn enhance bioremediation efficiencies. Organic pollutants introduced into the soil by military activities include energetics, chemical warfare agents and other military chemical compounds, generally grouped as potentially toxic compounds (PTC). Contamination by PTC on military bases is mainly caused by spillage of chemicals in storage areas (fuels, oils, lubricants, paints, solvents and corrosives). Their concentrations in the soil of military areas can be unacceptably high and, together with their high toxicity and persistence, can give rise to environmental hazards. The main objective of this study was to design a natural-based strategy for a soil contaminated with kerosene from a military air base (southern Spain), based on the combination of biostimulation and bioaugmentation in the presence of a model plant (sunflower, *Helianthus annuus*) and at the greenhouse scale (pots with 2 kg of soil). The bioaugmentation inoculant was a mixture of a motile strain (*Pseudomonas putida* G7), which is chemotactic towards sunflower root exudates, and a biosurfactant producing strain (*Bacillus subtilis* DSM10). This inoculant was designed to enhance the dispersion of the biosurfactant producer strain all over the soil volume in pots by chemotaxis-mediated co-mobilisation together with a mobile strain of *P. putida* G7. Microbial community response to treatments were assessed by 16S rRNA metabarcoding and qPCR targeting total (16S rRNA gene) and hydrocarbon-degrading (toluene dioxygenase gene, TOD) bacterial communities. The degradation of kerosene was favored in the presence of the plants. Biostimulation had a major effect on soil microbial community, clearly stimulating native aromatic hydrocarbon degrading populations (TOD). Bioaugmentation resulted in effective soil colonization by strain DSM10, that caused slight changes in microbial community structure between treatments.

4.06.P-Mo337 Metagenomic and Metatranscriptomic Analyses of a Bacterial Consortium for the Bioremediation of Total Petroleum Hydrocarbons Polluted Soil

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In order to design a bioremediation inoculant, a bacterial consortium was generated from a soil with a long story of hydrocarbon pollution in Noblejas (Toledo, Spain) by enrichment cultivation. Serial cultures of hydrocarbons polluted soil samples were grown in a minimal medium using diesel (1 mL/L) as the sole carbon and energy source. The bacterial composition of the Noblejas Consortium (NC) was determined by 16S rRNA gene amplicon libraries sequencing. The consortium contained around 50 amplicon sequence variants (ASVs), and the major populations belonged to the genera *Pseudomonas*, *Enterobacter*, *Delftia*, *Stenotrophomonas*, *Achromobacter*, *Acinetobacter*, *Novosphingobium*, *Allorhizobium*-*Neorhizobium*-*Rhizobium*, *Ochrobactrum* and *Luteibacter*. All other genera were below 1%. Metagenomic analysis of

NC has shown a high abundance of genes encoding enzymes implicated in aliphatic and (poly) aromatic hydrocarbons degradation, and almost all the pathways for hydrocarbon degradation were represented. Metagenomic analysis allowed the construction of high quality metagenome assembled genomes (MAGs) for the major players of NC. Metatranscriptomic analysis has shown that several of the ASVs are implicated in hydrocarbon degradation, being *Pseudomonas*, *Acinetobacter* and *Delftia* the most active populations. This consortium has been successfully used at microcosm (200 g) and pilot (700 kg) scales for Total Petroleum Hydrocarbons (TPHs) biodegradation. On the pilot scale, the combined use of the consortium plus biostimulation with vermicompost achieved 90.3% TPH removal after 90 days. The introduction of the consortium had a large impact on the polluted soil bacterial community, reflected by a decrease in alpha-diversity. Beta diversity analysis also showed that the bacterial community was different in the treated soil as compared with a control, untreated soil.

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4.06.P-Mo338 Shotgun Metagenomic Analysis of Microbial Community Profiles and Functions in Petroleum Hydrocarbon-Polluted Soils

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This study investigated the effects of petroleum hydrocarbon pollution on impacted soils of the Niger Delta. Petroleum pollution of the soil negatively impacts the microbial population, biodiversity, and activities within the area. The resistance of the indigenous microbial population to the toxicity of hydrocarbons is based on their energetic strategies and metabolic versatility in using the pollutant as carbon and energy source. In this study, shotgun metagenomics was used to analyze microbial diversity. Metabolic capability and functional profile of two petroleum hydrocarbon polluted sites in Bodo (N4.620134, 7.282998E) and Tombia (N04o47.714, E0006o51) communities, both in Rivers State Nigeria were investigated. Predominant phyla across all samples are the Proteobacteria. Other oleophilic phyla previously reported in oligotrophic environments like Acidiplilum, Actinobacteria, Verrucomicrobia, and Acidobacteria were found in the polluted soil. Stress response genes (cold shock, heat shock, starvation, osmotic stress, pH and oxidant detoxification) were well expressed in all samples analyzed. Genes for DNA repairs and de novo biosynthesis were also well expressed in addition to hydrocarbon degradation hydrogenases. Functional pathways include those for fermentation, respiration, de novo biosynthesis, and element cycling. These findings provide insight into the microbial activities and functional capabilities of indigenous microflora in petroleum hydrocarbon polluted soil.

4.06.P-Mo339 Assessing the Impact of Metals on Ecotoxicological Responses, Biomarkers, and Bioaccumulation in *Ceratophyllum demersum*: Implications for Phytoremediation Strategies

Santiago Martinez, Maria Elena Saenz, Jose Alberdi and Walter D. Di Marzio, PRIET DCB UNLU CONICET, Argentina

Phytoremediation processes for removing metals present in liquid effluents generally involve continuous exposure to relatively stable concentrations of these elements. These processes can be classified as tertiary treatments for effluents of industrial and municipal origin.

The removal of metals using aquatic plants is well-studied, although there are not many studies that employ the species *Ceratophyllum demersum* under conditions of continuous exposure from the discharge of a liquid effluent.

In this work, we define the sensitivity of this species to common metals found in discharges resulting from anthropogenic activities.

Acute ecotoxicity parameters, exposure biomarkers, and bioaccumulation kinetics are characterized based on exposure to solutions of each individual metal.

The results provide tools for monitoring the level of biochemical stress in the plants during the removal process, their tolerance to threshold concentrations, equilibrium times for reaching maximum accumulable concentrations (SST and MAC), and bioconcentration factors for each metal (BCF).

MAC (mg metal/g dry weight), steady state time (SST) to reach MAC in days and BCF for Ni, Zn and Cd were SST 14.14, 0.88, 4.24; MAC 0.87, 1.69, 0.15 and BCF 146, 210, 311.

The data obtained will contribute to the design of phytoreactors to be used for the removal of the studied metals and the biomonitoring of the plant fitness during remediation process.

4.06.P-Mo340 A Phytoremediation Mesocosm Using Aquatic Macrophyte Species at Different Plant Densities: First Results

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Release of heavy metals into the environment due to different activities are sources of metal contamination. Phytoremediation is a biobased technology, fundamental in the context of circular economy and sustainability.

The aim of this study was to develop a multispecies and multimetal mesocosm at lab scale, for the removing of heavy metals from a wastewater that discharge into Luján river (Bs Aires). The aquatic macrophytes chosen were *Lemna gibba*, *Ceratophyllum demersum* and *Elodea callitrochoides*. Plants were sampled from local streams and maintained in extensive cultures. The metals chosen were Cd, Zn and Ni as they are the most frequent in Luján river. Three different densities were assay (7, 15 and 30 grs/L of each specie). Different parameters were analyzed to determine which provided more efficient phytoremediation: removal rate, bioaccumulation factor, activities of antioxidant enzymes and whole ecotoxicity. Ecotoxicological assessments were done with *P. subcapitata* growth inhibition test and *D. magna* acute toxicity test. The experiments were set up for 10 days. Samples of multimetal solution were taken at time intervals. At the end of the experiments, an aliquot of plants was dried and digested for metal determination, while a subsample of fresh material was used to enzyme activities determinations. The density chosen was 15 grs/L as the selected criteria were better and the whole ecotoxicity of the phytoremediated solution was lower (Initial multi-metal ecotoxicity: *P. subcapitata* EC50-96hs: 1.3%; *D. magna* EC50-48hs: 6.4%; final phytoremediated solution ecotoxicity: *P. subcapitata* EC50-96hs: 57%; *D. magna* EC50-48hs: non ecotoxic). The experimental set up was repeated at this density but an inoculation with a microbial consortium from a wastewater bioreactor treatment plant was added to the system, as a strategy to enhance phytoremediation. Metagenomic analysis of this consortium was done by 16S rRNA sequencing analysis of V3-V4 region. A wastewater with metals that discharge to Luján river was treated under these two simultaneous conditions, resulting in a whole ecotoxicity removal and improvement of water quality parameters. The multispecies at selected density mesocosm in conjunction with inoculate with a microbial consortium demonstrated to be an excellent strategy to enhance metal removal.

4.06.P-Mo341 LIFE PFASTER (PFAS Systemic Regional Approach to Assess Spatial Distribution, Transfer, Exposure and Remediation of Widespread Pollution in Willebroek, Flanders)

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Extensive persistence of PFAS and its toxicity to life on earth, bioaccumulation, and adverse health and ecological effects make PFAS a top priority pollutant. Contemporary one substance, one assessment approaches to remediate PFAS are difficult to apply through the different compartments given the extensive group of precursors and metabolites that can arise in the environment. Current knowledge is insufficient to understand the risks posed by diffuse pollution to both people and the environment (one health approach) and has not yet been translated and integrated into management nor remediation practices. The recently started LIFE PFASTER (PFAS systemic regional approach to Assess Spatial distribution, Transfer, Exposure and Remediation of wide-spread pollution in Willebroek, Flanders) research project, aims to improve soil and water quality by developing a regional systemic remediation approach to reduce diffuse pollution with PFAS of soil, sediment, water and biota, including innovative, cost efficient methods to assess the spatial distribution and identification of exposure routes of the contaminants and the design and piloting of a replicable remediation approach beneficial for biodiversity and human health. Key steps (work packages in the project) include: 1) Assess composition, spatial distribution, transfer and ecological and related health risks of PFAS; 2) Design and pilot innovative and replicable (nature-based) remediation techniques, including phyto- and mycoremediation, mycofiltration, constructed and floating wetlands, in-situ flushing and physical-chemical groundwater treatment (including treatment trains with nanofiltration); 3) Develop a remediation approach and 4) Replicate the remediation approach and best practice techniques at other sites in Flanders and Europe.

Project area is a former industrial site (paper factory) located in Willebroek, Flanders (Belgium) and its surroundings, including a nature conservation area, an exemplary case for a heavily PFAS contaminated site and surrounding area. The project started in October 2024 and will run for 5 years. The 11 LIFE PFASTER expert partner organisations have a vast network of local, regional, (inter)national partners for project input, dissemination and replication. See for more info: <https://ovam.vlaanderen.be/nl/w/naar-een-gebiedsgerichte-en-natuurgebaseerde-aanpak-van-pfas-verontreiniging-in-vlaanderen-met-de-steun-van-europa?redirect=%2Fnieuws> and soon www.lifepfaster.eu

4.06.P-Mo342 Remediation of PFAS Contaminated Soil using the Novel Combination of Biochar Sorbent Stabilization and Phytoaccumulation: First Insights

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Biochar is a carbonous, porous material produced by pyrolysis of biomass, which can be potentially used as a sorbent for contaminants and nutrients in soil and water. In recent years, biochar has increasingly been put forward as an amendment that can be used for soil remediation, whereby contaminants are stabilized and ecological restoration of soil is promoted. This could especially be a solution for larger areas with low- to medium level contaminated soil outside source zones. As a positive side effect, addition of biochar could also enhance the carbon content and soil quality of degraded soils.

Two potential effective (bio)char types for remediation of soils contaminated with per- and polyfluoroalkyl substances (PFAS) are activated carbon (AC) and sewage sludge-based biochar (BC-SS). Both have demonstrated similar, large sorption capabilities for longer chain PFAS, however, sorption of shorter chain PFAS may be less effective. Furthermore, the profound effects that these sorbents can have on soil organisms also remain an area requiring further research.

A potential solution may be a combined approach of sorbent stabilization with phytoaccumulation, where shorter chain, more mobile PFAS may be taken up by plants for subsequent removal and destruction. In this study, this novel in situ remediation concept combining sorbent stabilization and phytoaccumulation is tested. Additionally, the toxicological and ecological effects on soil organisms are also assessed, complementing the PFAS sorption and mobility study.

We added 2% of either a wood-based, commercially available AC or a SS-BC to PFAS contaminated sandy loam soil, together with 1% compost and inorganic fertilizer. These mixtures were tested for PFAS leaching, uptake in perennial ryegrass and earthworms, and effects on the grass, earthworms and the soil microbiological activity and community composition. This presentation will present the first insights on comparing the effectiveness of the two biochar types in this combined sorbent-phytoaccumulation approach, as well as compare the effects and uptake between different soil biota. This information is of vital importance, as the potential usage of biochar-remediated low-to medium contaminated areas is most likely urban parks where not only the contaminant stabilization, but also the ecological soil quality has to be taken into account.

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4.06.P-Mo343 What Biochar Properties Determine Sorption Strength of Perfluorooctanoic Acid (PFOA) to 23 Biochars Produced from Various Organic Waste Types?

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Biochar has the potential to be a sustainable sorbent for per- and polyfluoroalkyl substances (PFAS). However, what biochar properties influence PFAS sorption are still poorly understood. Here, we produced twenty-three biochars from sewage sludge, food waste reject, wood wastes, and one reference substrate (wood pellets), at various temperatures (500-800 °C) using two pyrolysis technologies. The purpose of this study was to fill the knowledge gaps on and develop a better understanding of which waste-derived biochar properties most strongly influence PFAS sorption. The biochars were characterized for pore volume, surface area, elemental content, ash content, pH, zeta potential, condensed aromatic carbon (ConAC) and their -OH functional group content. Sorption isotherms of perfluorinated octanoic acid (PFOA) was determined by the Polanyi-Dubinin-Manes (PDM) and Freundlich models, and the sludge-based biochars outperformed the wood-derived biochars. Multiple linear regression models were developed to couple a selection of biochar properties to sorption of PFOA. The capacity (V0), and affinity terms (E) from the PDM model, and the capacity (nF) and affinity (log KF) terms from the Freundlich model were chosen as response variables.

A high porosity in pores with diameters (3-6 nm), about twice the molecular dimensions of PFOA, was identified as the key factor governing sorption capacity. The greater sorption capacity of PFOA to the sludge biochars was attributed to their mesoporous structure, whereas the wood biochars predominately consisted of micropores being inaccessible for PFOA due to steric hindrance. Sorption affinity was likely governed by hydrophobic interactions between PFOA and aromatic carbon-rich regions, especially for the wood biochars. Our results show that various organic waste can serve as feedstock for production of biochar for soil and water treatment. The insights gained in this study can improve the understanding of which parameters that are most important for PFAS sorption and thereby guide pyrolysis conditions and selection of feedstocks towards production of high-quality biochars with physicochemical properties tailored to its intended application.

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4.06.P-Mo344 Potentially Toxic Elements Distribution and In Vitro Bioaccessibility at House Fire Impacted Sites on Gitxsan Territory (Hazelton), British Columbia, Canada

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Potentially toxic elements (PTEs) may be present in soil after a house fire. The human health and ecological risk associated with the PTEs may be elevated and as such soil remediation may be required to restore the site for rebuilding. The Skeena Watershed Conservation Coalition (SWCC), a non-profit environmental organization located in Hazelton, BC on the unceded and traditional territories of the Gitxsan First Nations, is exploring the development of regenerative and community-based ecological solutions that support community resilience and a healthy watershed for future generations in a culturally appropriate manner. Recent structural fires have burned down houses in the Gitxsan communities of Gitwangak, Gitanmaax and Sik-E-Dakh. These sites pose a contamination threat to the community, as well as serve as a continual reminder of trauma. In partnership with the Gitxsan First Nation communities, SWCC is investigating bioremediation as a potential remedial option for these house fire impacted areas. Characterization of the contaminants present at these sites is an essential first step. This study was initiated with a workshop in each of the First Nation communities to engage members in the planning, sampling, conducting remediation and follow up monitoring. Potential contaminants of concern arising from house fires were also discussed. After completion of the workshop, First Nations participants assisted in collecting 74 soil samples from five house fire impacted sites. The samples were analyzed for PTEs by x-ray fluorescence. PTEs including arsenic, chromium, copper, lead and zinc in some of the samples exceeded Canadian Environmental Soil Quality Guidelines for residential land use. Ongoing work includes determining soil pH, in vitro bioaccessibility and the potential human health risk associated with exposure to the PTEs using Health Canada guidelines incorporating receptor characteristics for Canadian First Nation Populations. Recommendations on limiting exposure to contaminated soils at each of the house fire sites will be developed. Remedial options analysis will be conducted in collaboration with SWCC and the Gitxsan community members to determine the most suitable remedial technique. If the communities are interested in further remedial action, it is possible that the sites could become the focal

points of action for future community-based hands-on bioremediation application and learning opportunities that incorporate indigenous knowledge systems.

4.07.A Advancing Treatment of Organic Micropollutants in Water and Wastewater

4.07.A.T-01 Integrated Assessment of Biological Activated Carbon Filters with UV/Peracetic Acid Pretreatment for Mitigating Organic Micropollutants and Toxicity in Treated Wastewater

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The presence of organic micropollutants (OMPs) in wastewater poses a significant environmental concern, affecting humans and aquatic ecosystems even at trace levels. With increasing population and climate-driven water shortages, wastewater reuse strategies are gaining importance. This study evaluated the efficacy of biological activated carbon (BAC) filtration in combination with UV/peracetic acid (UV/PAA) pretreatment for OMP removal and toxicity reduction in treated wastewater. UV/PAA pretreatment was compared to UV/H₂O₂, assessing the removal efficiency of key OMPs and overall water toxicity via chemical analysis and bioassays for estrogenicity, cytotoxicity, phytotoxicity, and oxidative stress. The results indicated that UV/PAA pretreatment was more effective than UV/H₂O₂ in degrading a wide range of OMPs, including trimethoprim (44.5%), venlafaxine (42.5%), diuron (52%) and diclofenac (100%). UV/PAA also significantly reduced photosynthesis inhibition (53%) compared to UV/H₂O₂ (21%). Biofiltration following UV/PAA reduced algal toxicity by 63% and lowered the estrogenic load by over 60%, bringing estrogenicity risk from high to moderate. While both treatments reduced bacterial toxicity caused by advanced oxidation processes, biofilters treated with UV/H₂O₂ pretreated water showed a greater reduction (25%) than those with UV/PAA pretreatment (3%). Microbial community analysis revealed that biofilters supported the growth of OMP-degrading bacteria such as *Pseudomonas*, *Mycobacterium*, and *Rhodococcus*. UV/PAA pretreatment facilitated the growth of species such as *Acinetobacter* and *Anaerolinea* that have the capability of degrading estrogens and other OMPs, indicating its effectiveness in mitigating toxicity. This study provides valuable information on the synergistic combination of UV/PAA oxidation with biofiltration, in degrading OMPs and mitigating toxicity in treated wastewater.

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4.07.A.T-02 Micropollutant Mass Balance in an Integrated System Coupled by Biological Treatment and Nanofiltration with Recirculation

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Conventional wastewater treatment plants (WWTP) are not designed to completely remove organic micropollutants (OMPs), due to the low concentration in influent wastewater, limited contact time within the system (from 8-12 h) and some OMPs being persistent to biodegradation. Nanofiltration (NF) may be used to retain OMPs from treated effluents. NF is suitable to remove OMPs from 200-2000 Da, it has a lower consumption of energy compared to reverse osmosis and classical ozonation, and it does not generate unwanted byproducts, except a concentrate stream.

Here we explore the combination of conventional wastewater treatment with NF to enhance the overall OMP removal. Recirculating the concentrate stream (retentate) from the NF system into the biological treatment might increase the concentration and contact time of OMPs within the biological system and hence reduce their mass flux in the treated effluent.

We have observed that the concentrate stream from the NF system can be treated in the biological system. Negatively charged compounds and larger molecules are being retained and upconcentrated in the bioreactor. The mass flux of irbesartan, sulfamethoxazole and bezafibrate in the effluent was decreased by 3.9, 5.1 and 4.7 times following NF re-circulation. Salts from the concentrate in terms of sulphates and phosphates did not affect the microbial activity of the sludge in the integrated system. Thus, a concentration of 90% NF recovery is feasible in a continuous integrated system. Our work unravels mass fluxes of OMPs with real water matrices and a novel integrated system that couples SBR and NF to decrease the release of OMPs from WWTP effluents in the environment. This work can inform the

planning and implementation of future integrated WWTP setups to comply with the new European urban wastewater treatment directive.

4.07.A.T-03 Removal of Organic Micropollutants Under Dry and Wet Weather in a Full-Scale Aerobic Granular Sludge Plant

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Aerobic granular sludge (AGS) is an effective wastewater treatment widely applied worldwide for the removal of nutrients and organic matter. However, limited information is available on the effectiveness of full-scale AGS plants for removing organic micropollutants (OMP), particularly during wet weather. This study investigated the occurrence and removal of OMPs, including 19 pharmaceuticals and 2 industrial compounds, in a full-scale AGS plant during both dry and wet weather over one year.

We selected a full-scale AGS plant located in Utrecht, the Netherlands as the targeted AGS plant, and collected monthly 24-hour composite water samples and grab sludge samples from May 2023 to April 2024. 21 OMPs were extracted from water and sludge samples and measured by LC/MS. Concentrations of ammonia and organic matter (both particulate and soluble) were measured with HACH Lange GMBH kits.

The results show that Influent concentrations of 5 pharmaceuticals and 1 industrial compound exceeded 1 µg L⁻¹, which were diluted by rainfall. Influent loadings of 7 OMPs (absolute OMP amount in µg day⁻¹) were significantly increased during wet weather, likely from sewage sediment resuspension and urban runoff. Average removal efficiencies of 11 compounds achieved greater than 20%, with 5 of them exceeding 50%, in the AGS plants. Simple linear regression results between OMP removal efficiencies and flow rates showed that biodegradable OMPs were more strongly affected by increased influent volumes (as indicated by steeper slopes) than OMPs primarily removed through sorption. Additionally, correlation analysis results showed that the removal of soluble organic matter was significantly correlated (p-value < 0.05) with the removal of 14 OMPs, suggesting that organic matter removal may be an indicator for OMP removal in the AGS plant. After AGS treatment, 8 compounds in the effluent remained above their predicted no-effect concentration levels, indicating potential ecological and human health risks in the receiving water. During wet weather, effluent OMP loadings increased mainly driven by reduced removal efficiency. Additionally, compared to activated sludge plants, the AGS plant exhibited comparable or slightly higher OMP removal efficiency during dry weather.

Overall, this study is the first to investigate OMP removal during wet weather in a full-scale AGS plant and propose the potential impact of increased flow rates on biodegradable OMPs or OMPs mainly removed through sorption.

4.07.A.T-04 Integrating Foam Fractionation into the Activated Sludge Process for Per- and Polyfluoroalkyl Substances Removal from Landfill Leachate: An Energy-Efficient Approach

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Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals known for their persistence, bioaccumulation, and toxicity. Landfill leachate is a major source of PFAS contamination in the environment. However, conventional wastewater treatment plants (WWTPs) are not designed to effectively remove PFAS, highlighting the need for specialized treatment technologies. Foam fractionation (FF) has shown promise in removing PFAS from landfill leachate, achieving removal efficiencies up to 60%. FF is considered a sustainable method, as it does not require chemical reagents or adsorbents. However, energy for aeration impacts its cost and energy efficiency.

This study investigates integrating FF into the activated sludge (AS) process, offering a more energy- and cost-efficient approach to mitigate PFAS emissions. AS processes already rely on aeration to treat nutrients and organic matter, and foam formation frequently occurs due to the presence of surfactants and filamentous bacteria. While foam formation is typically suppressed to avoid operational challenges, this study investigates its potential use for PFAS removal.

Laboratory experiments were conducted using a bench-scale activated sludge reactor treating landfill leachate. In the first phase, foam formation was suppressed with an antifoam agent to mimic conventional operations. This ensured that PFAS removal could occur solely through sorption to sludge. In the second phase, no antifoam was added, allowing natural foam formation and its subsequent removal. The analysis involved the general chemistry and the concentrations of 32 different types of targeted PFAS, in the

influent, effluent, and foam. The PFAS analyses were performed using Solid Phase Extraction (SPE) followed by LC-MS/MS.

Results indicated negligible PFAS removal during the first phase, while the second phase achieved removal efficiencies comparable to standalone foam fractionation. However, also solids were enriched in the foam, potentially impacting the microbial population in the bulk solution. Preliminary observations suggest no significant difference in biological performance between the two phases. These findings offer promising insights for WWTP operators seeking cost-effective strategies to mitigate PFAS emissions. Given the widespread use of activated sludge reactors in municipal and industrial wastewater treatment plants, integrating foam fractionation into these processes presents a potential scalable solution.

4.07.A.T-05 Chemical and Toxicological Evaluation of Advanced Oxidation Treated Pharmaceutical Residues in Wastewater

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Pharmaceuticals are commonly used to prevent and treat diseases or alleviate pain in human and veterinary healthcare. Drugs are primarily discharged via domestic and hospital sewage water (HSW) for removal by wastewater treatment plants (WWTPs). WWTPs are not designed for the removal of these complex chemicals, leading to release into the aquatic environment and negatively impacting biodiversity. To complement WWTPs and prevent incomplete removal of unwanted pharmaceuticals, on-site wastewater treatment can contribute to mitigation for this problem. This study aimed to assess the effectiveness of on-site oxidative treatment of hospital sewage water (HSW) using advanced oxidation processes (AOPs). Thermal plasma and UV-C/H₂O₂ oxidation technology were applied on surrogate matrices spiked with 14 drugs (iopamidol, iomeprol, diatrizoic acid, fluoxetine, diclofenac, metoprolol, cyclophosphamide, carbamazepine, terbutaline, phenazone, acetaminophen, ciprofloxacin, doxycycline and metformin). In addition, untreated end-of-pipe HSW was analysed and subjected to oxidation. All drugs were partly or completely decomposed by thermal plasma and UV-C/H₂O₂ treatment. In HSW, 10 pharmaceuticals were quantified ranging in concentrations from 0.08 to 2400 µg/L. When AOP was applied, it was found that overall pharmaceutical degradation in HSW was nearly equivalent to results found in surrogate matrices. Subsequently the effectiveness and safety of AOP treatment was evaluated using a HeLa cytotoxicity assay and in silico environmental risk assessment. Acute cytotoxicity of untreated HSW was reduced after oxidation. Quantitative structure-activity relationship modelling (QSAR) of aquatic toxicity demonstrated good concordance with the cytotoxicity findings. We have shown that AOPs can be considered promising technologies to remove medicinal residues from hospital wastewater.

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4.07.B Advancing Treatment of Organic Micropollutants in Water and Wastewater

4.07.B.T-01 Which Factors Limit the Adsorption of Per- and Polyfluoroalkyl Substances (PFAS) on Activated Carbon During Drinking Water Treatment?

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Per- and polyfluoroalkyl substances (PFAS) are persistent micropollutants commonly detected in surface and groundwater at low ng/L concentrations. The current EU directive limits PFAS in drinking water to 100 ng/L for a mixture of PFAS compounds of concern, with a proposed reduction to 4.4 ng/L. Although activated carbon adsorption is widely used for PFAS removal, rapid breakthrough in activated carbon filters limits effectiveness, requiring frequent regeneration and raising treatment costs. Most studies on PFAS adsorption have been conducted at higher concentrations, making it challenging to predict performance under environmentally relevant conditions.

This study aims to identify factors limiting PFAS adsorption on AC in drinking water treatment, focusing on low ng/L concentrations. We tested a mixture of short- and long-chain PFAS compounds on widely used activated carbons with different pore size distributions. Batch (isotherm and kinetic) and rapid small-scale column tests were conducted under varying PFAS concentrations, temperatures, and organic matter fractions. For the mentioned conditions we report experimentally obtained surface diffusion coefficients and isotherm data which we use to predict PFAS breakthrough.

Results show that data from higher PFAS concentrations significantly overpredicts adsorption at ng/L levels which is likely a consequence of multilayer PFAS adsorption at high concentrations. Temperature increases from 10 to 30 °C decreases short-chain PFAS adsorption by several times, whereas long-chain PFAS adsorption is unaffected. Smaller organic matter fractions (<3 kDa) inhibit short-chain PFAS adsorption, while the hydrophobic neutral fraction strongly limits long-chain PFAS removal. Our findings suggest that optimizing the removal of specific organic matter fractions before activated carbon treatment can significantly enhance PFAS adsorption efficiency. For treatment plants focused on removing short-chain PFAS, operators should account for seasonal temperature variations, as these can impact removal effectiveness. This study offers valuable insights to improve PFAS removal in drinking water treatment, supporting alignment with proposed regulatory standards.

4.07.B.T-02 Polymer Composite Membrane Processes for Treatment of Contaminants of Emerging Concern in Effluents of Urban Wastewater Treatment Facilities

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Freshwater is increasingly threatened by pollution and diminishing supplies, particularly from industrial wastewater and municipal sewage. Wastewater treatment plants (WWTPs) use biological processes to purify sewage before discharge into surface waters. However, treated effluents often contain contaminants of emerging concern (CEC), such as pharmaceuticals, personal care products, drugs, and steroid hormones, which pose risks to aquatic organisms and drinking water sources. Various treatment methods have been explored, including activated carbon filtration, advanced oxidation processes (AOPs), membrane filtration, chemical precipitation, ion exchange, adsorption, phytoremediation, electrochemical treatment, and source control. However, many of these methods suffer from limitations such as low efficiency or high costs. As freshwater availability decreases in regions like Central Asia, there is an urgent need for low-cost, reliable, and robust wastewater treatment methods for the safe reuse of treated wastewater, especially for irrigation and other uses. Catalytic wet peroxide oxidation (CWPO) is a promising advanced oxidation process for degrading organic pollutants under mild conditions, using inexpensive clay- and carbon-based catalysts. Membrane technologies also show potential for CEC removal, offering high separation efficiency. Combining polymers with clay- or carbon-based materials enhances removal through filtration and adsorption. This study quantified CECs in southern Kazakhstan using polar organic chemical integrative samplers (POCIS) and proposed solutions using polymer-mixed composite membrane-based processes. These low-cost, sustainable membranes present an environmentally friendly option for wastewater treatment. Sampling surface waters in the south region of Kazakhstan, dominated by the discharge of effluents from urban WWTP, identified 16 CECs, including caffeine, 1,7-dimethylxanthine, paracetamol, cotinine, and sulfamethoxazole (SMX). To present solutions for the removal of CECs, polymer-mixed composite membrane processes were tested considering organoclays and MnFe₂O₄ clays produced from natural clays, to be used as filler materials in the membrane processes. The composite polymeric clay-based membranes have shown promising results, with the incorporation of Shymkent organoclay and MnFe₂O₄/Turkistan clay proving high adsorption of SMX used as the model pollutant.

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4.07.B.T-03 Enhancing Wastewater Quality through SAT Systems: Assessing the Effectiveness of Natural Reactive Barriers for Contaminants Removal

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Water scarcity and the presence of contaminants of emerging concern (CECs) in treated wastewater pose significant challenges to water reuse strategies. This study evaluates the effectiveness of a Soil Aquifer Treatment (SAT) system with reactive barriers in removing CECs from urban wastewater treatment plant effluent used for aquifer recharge in Palamós, Spain. We compared two SAT systems: a control filled with sand (B2) and one incorporating a reactive barrier (B3) composed of sand, organic matter, biochar, zeolite, and clay, with samples collected at depths of 15, 28, 41 and 54 cm. Using High-Resolution Mass Spectrometry (HRMS) with Non-Target Analysis (NTA), we analyzed water and barrier samples over a 73-day period. Results showed that B3 exhibited higher CEC detection (320 compounds) compared to B2 (212 compounds), attributed to enhanced adsorption and surface area. Water analysis revealed lower CEC numbers in both systems post-treatment, particularly in B3, indicating more effective removal. However, some compounds increased after treatment, especially in B3, suggesting potential metabolite or transformation product formation within the reactive barrier. The study demonstrates the potential of reactive barriers in SAT systems for enhancing water quality and addressing water scarcity, while highlighting the need for further research on barrier optimization and transformation product formation. These findings contribute to advancing treatment strategies for organic micropollutants in water reuse application.

4.07.B.T-04 Removal of Antibiotics and Nutrients by Microalgae-Bacteria Consortia

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The persistence of pharmaceutical contaminants in aquatic ecosystems has raised significant environmental concerns due to the accumulation of antibiotics in water bodies. Traditional wastewater treatments often fail to remove these contaminants fully, so exploring alternative bioremediation strategies is essential. The current study investigates the potential of co-cultivating *Chlorella sorokiniana* and bacteria isolated using an iChip system to enhance the removal of antibiotics and nutrients from contaminated water. Laboratory experiments assessed the removal efficiency of ten antibiotics Penicillin V (PenV), Oxytetracycline (OTC), Amoxicillin (AMX), Cephalexin (CEX), Ciprofloxacin (CIP), Metronidazole (MTZ), Clarithromycin (CLR), Azithromycin (AZM), Sulfamethoxazole (SMX), and Trimethoprim (TMP) alongside nutrient analysis for ammonium, nitrate, phosphate, and chemical oxygen demand (COD) over 21 days.

The co-cultivation treatments achieved high % removal for key antibiotics, with PenV, OTC, AMX, and CEX showing removal efficiencies above 90%, while moderate removal rates were recorded for AZM, CIP, and CLR (up to 50%), and lower rates for MTZ, SMX, and TMP. Nutrient removal in the co-culture reached 96% for COD, 32% for nitrate, and 100% for phosphate after 21 days. This suggests that the co-culture system facilitated efficient nutrient recycling, possibly through mutualistic interactions where microalgae provided oxygen to enhance bacterial degradation, and bacteria supplied CO₂ for algal photosynthesis. Bacterial community analysis further highlighted significant shifts in microbial composition in the presence of algae and antibiotics.

The results of microalgae-bacteria consortia effectively removing antibiotic contaminants and nutrients suggest that this sustainable approach could be integral to future water treatment and management systems. This study contributes valuable insights into optimising bioremediation strategies for pharmaceutical and nutrient contaminant removal in aquatic environments, positioning microalgal-bacterial systems as an efficient solution to improve water quality and mitigate pharmaceutical pollution.

4.07.P-Mo349 Biotransformation and Biodefluorination of n:3 Fluorotelomer Acids in Sludge-Derived Enrichment Culture

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The extensive use of per- and poly-fluoroalkyl substances (PFAS) in various industries has led to significant environmental contamination, particularly from fluorotelomer-based aqueous film-forming foams (AFFF). This study investigates the biodefluorination capabilities of a unique microbial consortium derived from activated sludge, focusing on its ability to transform n:3 fluorotelomer carboxylic acids (n:3 acids). Previous research identified one-carbon removal pathways as key mechanisms for the stepwise biodegradation and defluorination of select fluorotelomers; however, an enrichment culture and a comprehensive understanding of these biotransformation processes were lacking.

We maintained this enrichment culture over 13 generations, demonstrating sustained defluorination efficiencies of 17% for 5:3 acid and 15% for 4:3 acid. In a detailed experiment conducted over one generation, we incubated the culture for up to 62 days. The culture exhibited logarithmic growth and stable pH, with notable reductions in initial acid concentrations of 46% and 84% after 10 days during the exponential growth phase. Additionally, we explored the unusual incorporation of 5:3 acid into biomass

during the logarithmic growth phase, revealing that 90-97% of the acid was retained during exponential growth. The differential results from two extraction methods suggest a reversible integration mechanism likely involving covalent bonding rather than simple biosorption.

Transformation products, including shorter-chain n:3 acids, short-chain perfluoroalkyl acids and other intermediates, were identified using ultra-high performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS), underscoring the complexity of PFAS biotransformation pathways. Our metagenomic analysis further elucidates the microbial communities involved in defluorination, providing valuable insights into potential defluorinating enzymes and metabolic pathways. While we have yet to fully identify the key microbial players responsible for effectively degrading these highly fluorinated compounds, continued analysis of the metagenomic data promises to uncover critical information for future investigations. These findings enhance our understanding of natural attenuation processes and inform the potential for the biological treatment of polyfluorinated chemicals.

4.07.P-Mo353 Sorption and Biodegradation of Organic Micropollutants in Aerobic Granular Sludge

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Aerobic Granular Sludge (AGS) is a relatively new approach to wastewater treatment. As opposed to activated sludge with mainly flocculant biomass of relatively even composition, the granules in AGS differ in size and composition. Biomass ranges from flocs similar to activated sludge to granules of >6 mm. Granules also have distinct biomass due to the stratification of redox conditions across the granules. Typically organic micropollutants (OMPs) are removed via sorption and biodegradation during wastewater treatment, however this has yet to be thoroughly investigated for AGS systems. We hypothesized that different sized granules have potentially different sorption behaviour due to the differences in extracellular polymeric substances (EPS) and have different biodegradation patterns due to the differences in biomass composition among size fractions. Therefore, we investigated the capacity of AGS to remove OMPs via sorption and biodegradation by performing controlled batch experiments in the lab. We separated the sludge into different sizes in order to understand the role of different sludge composition on OMP removal.

We observed notable sorption (>40% removal) for 10 of the 24 OMPs tested in our study (Figure 1). The 10 OMPs include 3 fluoroquinolones (norfloxacin, ofloxacin, and ciprofloxacin), 3 macrolides (clarithromycin, azithromycin, and erythromycin), 2 beta-blockers (propranolol and atenolol), tetracycline, and citalopram. We noted that all of these 10 compounds are ionizable, with 6 positively charged compounds and 4 zwitterionic compounds at pH 7. Considering that sludge biomass typically has a negative charge, this seems to indicate electrostatic interactions between the 10 OMPs and the sludge. Larger fractions contributed more to sorption than smaller granules and flocs, as expressed per unit of biomass. Specifically, the normalized K_d for large fractions was up to 100% larger than for small fractions, suggesting that larger fractions contribute more to sorption in real AGS systems than smaller fractions and flocs. However, sorption kinetics were most likely retarded by diffusion limitations in large granules. Biodegradation was observed for a number of compounds. Overall, this study elucidated the roles of sorption and biodegradation in the removal of OMPs in AGS WWTPs, showing that these processes are size fraction dependent.

4.07.P-Mo362 Phytotoxicity as a Tool for Evaluating Toxin Removal from Industrial Waste Following EGSB Reactor Operation

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The wet coffee bean processing generates two main wastes: solid lignocellulosic material (husk and pulp) and wastewater. With Brazil producing 3.6 million tons of coffee annually, this results in 27 to 432 million tons of solid waste and 1.8×10^9 to 5.4×10^9 liters of wastewater. Coffee waste is rich in carbohydrates and organic matter but contains toxic polyphenols, leading to environmental issues like aquatic eutrophication if improperly disposed of. Anaerobic digestion presents a promising solution by reducing organic matter and producing bioenergy (CH₄). Combining coffee residues with brewery wastewater can

enhance biogas production, as polyphenols are diluted, improving organic matter utilization. Brewery wastewater production is substantial, with 10-30 m³ of water used per ton of beer, yielding 10-20 m³ of wastewater. Expanded Granular Sludge Bed (EGSB) reactors are effective for wastewater treatment, enhancing COD removal through bed expansion, improving organic matter reduction. To confirm the efficiency of anaerobic digestion in reducing toxicity, phytotoxicity studies provide a reliable assessment tool. This study investigated the effluent from an EGSB reactor using phytotoxicity assays to evaluate its efficiency in treating the combined wastes.

4.07.P Advancing Treatment of Organic Micropollutants in Water and Wastewater

4.07.P-Mo345 Characterization and Removal of Contaminants of Emerging Concern (CECs) During the Biological Treatment of Wastewater Batches from Individual Wastewater Treatment Systems

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Approximately 11% of the European Union population is not connected to sewage collection systems, especially in rural areas (Eurostat 2019), which can be a source of diffuse pollution. Among individual wastewater treatment/collection systems, septic tanks and holding tanks are widely used in Spain. Wastewater and sludge from these systems exhibit higher COD, total nitrogen (TN) and phosphorus concentrations compared to municipal sewage. Moreover, Information regarding contaminants of emerging concern (CECs) in these streams is scarce.

In this study, wastewater batches purged from holding tanks were treated using two lab-scale systems: an Upflow Anaerobic Sludge Bed (UASB) reactor and a Conventional Activated Sludge (CAS) reactor, both operated for almost 90 days under a hydraulic retention time of 1.37 ± 0.51 days. The average inlet COD and TN concentrations were 968 mg COD/L and 142 mg N/L, which are approximately twice as high as those typically found in urban wastewater. COD removal efficiencies were quite similar in both reactors ($73 \pm 7\%$ in CAS vs. $76 \pm 10\%$ in UASB). Nitrification-denitrification was achieved in the CAS unit by alternating cycles of aeration and anoxia, being achieved a total nitrogen removal of $27 \pm 13\%$, with nitrate as the predominant N form in the effluent.

Seven antibiotics, erythromycin (ERY), clarithromycin (CLA), roxithromycin (ROX), azithromycin (AZY), sulfamethoxazole (SMX), trimethoprim (TMP), and ciprofloxacin (CIP), were detected in concentrations of 0.1-10 µg/L, with CIP and AZY exhibiting the highest values. Anti-inflammatories ibuprofen (IBP), naproxen (NPX) and diclofenac (DCF) were also detected, with IBP reaching up to 35 µg/L. Removal varied under different reactor conditions: under aerobic/anoxic (CAS) five CECs were removed beyond 70% (ERY, ROX, SMX, IBP and NPX), while under anaerobic conditions only three were effectively removed (ROX, TMP and NPX). DCF is the most recalcitrant compound and the macrolide AZY was removed to a very low extent in both reactors.

These findings highlight the challenges posed by wastewater management in rural areas, which should be addressed taking into account all the complexity present in these streams, particularly due to the presence of CECs.

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4.07.P-Mo346 Wide-Scope Suspect Screening of 24 Sewage Treatment Plants

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Sewage treatment plants (STP) affect water quality by discharging effluent by emitting nutrients and micropollutants. We will present measurements on 24 STPs in The Netherlands on both influent and effluent using wide-scope suspect screening. Our aim was to include known substances, emerging substances and (potential) Substances of Very High Concern. Influent mainly contained substances of human origin, sweeteners, medicines and industrial substances. In total, approximately 15,000 substances were found, of which 353 substances have been identified with high confidence (level 1-2). Of these, 168 substances are linked to a category that indicates the origin. Relatively speaking, medicines are the most frequently detected (118 substances), followed by industrial and household substances (39) and a small group of pesticides, including biocides (11). Six 'Substances of Very High Concern' and four potentially

Substances of Very High Concern have been determined: such as PFBS, a type of PFAS and tetraglyme (solvent). Some drug metabolites may be formed in WWTPs. All WWTPs seem to have many similarities in the disposal of substances, while the differences may be explained by using additional information.

4.07.P-Mo347 Understanding the Influence of Micro and Nano Plastics (PP, PE, and PS) on MBR Performance: A Comprehensive Study

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Introduction. Microplastics and Nanoplastics (MNPs) pose a pressing environmental concern as they inevitably infiltrate wastewater treatment plants (WWTPs), spreading into aquatic and terrestrial ecosystems through water reuse and sludge application. Membrane Bioreactors (MBRs) have demonstrated high efficiency in removing MPs, achieving rates as high as 99.9%. However, significant gaps persist in understanding the removal dynamics of MNPs and their long-term effects on MBR performance. While studies have examined individual MNPs, the combined impact of mixed plastics such as polyethylene (PE) and polypropylene (PP) microplastics with polystyrene (PS) nanoplastics remains largely unexplored. In this context, this study seeks to fill this critical research gap by investigating the removal efficiency and the synergistic impacts of the accumulation of these mixed plastics on water quality, the floc characteristics, microbial community, and fouling behavior, within a submerged integrated aerated membrane bioreactor system.

Experimental/methodology. The experiments were conducted in a laboratory scale fully automatic aerobic MBR that contains two independent tanks and an effective flat sheet membrane. The systems were seeded with aerobic sludge that was collected from the aeration basin of a WWTP located in Guadalajara (Spain) and was daily fed with synthetic wastewater. One reactor was used as a control, and the other one contained the addition of NP-PS, MP-PP and a recycled MP-PE. The study spans 120 days, including an initial acclimatization phase.

Results and discussion. Preliminary findings revealed that both MBR systems achieved high organic removal efficiencies, with COD reduction exceeding 97%. MP accumulation influenced the microbial structure and diversity, with the microbial community adapting over time under MNP stress. Moreover, MPs demonstrated a potential scouring effect on the membrane, as indicated by trends observed in the TMP profile analysis. Further analyses, including EPS characterization, detailed membrane fouling quantification, and MNP assessment results will provide a more comprehensive understanding of these interactions.

These analyses will contribute to a comprehensive understanding of the interactions between MNPs and the biological and physicochemical components of the treatment process, thereby informing effective mitigation strategies and advancing our knowledge of MNPs removal in MBRs wastewater treatment systems.

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4.07.P-Mo349 Biotransformation and Biodefluorination of n:3 Fluorotelomer Acids in Sludge-Derived Enrichment Culture

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The extensive use of per- and poly-fluoroalkyl substances (PFAS) in various industries has led to significant environmental contamination, particularly from fluorotelomer-based aqueous film-forming foams (AFFF). This study investigates the biodefluorination capabilities of a unique microbial consortium derived from activated sludge, focusing on its ability to transform n:3 fluorotelomer carboxylic acids (n:3 acids). Previous research identified one-carbon removal pathways as key mechanisms for the stepwise biodegradation and defluorination of select fluorotelomers; however, an enrichment culture and a comprehensive understanding of these biotransformation processes were lacking.

We maintained this enrichment culture over 13 generations, demonstrating sustained defluorination efficiencies of 17% for 5:3 acid and 15% for 4:3 acid. In a detailed experiment conducted over one generation, we incubated the culture for up to 62 days. The culture exhibited logarithmic growth and

stable pH, with notable reductions in initial acid concentrations of 46% and 84% after 10 days during the exponential growth phase. Additionally, we explored the unusual incorporation of 5:3 acid into biomass during the logarithmic growth phase, revealing that 90-97% of the acid was retained during exponential growth. The differential results from two extraction methods suggest a reversible integration mechanism likely involving covalent bonding rather than simple biosorption.

Transformation products, including shorter-chain n:3 acids, short-chain perfluoroalkyl acids and other intermediates, were identified using ultra-high performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS), underscoring the complexity of PFAS biotransformation pathways. Our metagenomic analysis further elucidates the microbial communities involved in defluorination, providing valuable insights into potential defluorinating enzymes and metabolic pathways. While we have yet to fully identify the key microbial players responsible for effectively degrading these highly fluorinated compounds, continued analysis of the metagenomic data promises to uncover critical information for future investigations. These findings enhance our understanding of natural attenuation processes and inform the potential for the biological treatment of polyfluorinated chemicals.

4.07.P-Mo351 Can Wastewater Treatment Plants' Mitigate Microplastic Contaminations? – A Comparative Study of Sludge Treatments.

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The primary objective of this study is to quantify the efficiency of various sludge treatment methods in removing microplastics (MP) from sewage sludge at wastewater treatment plants (WWTPs). Given that approximately 99% of MPs entering WWTPs are retained in the sludge fraction, understanding the effectiveness of these treatments is critical for mitigating the environmental risks associated with the application of sludge as fertilizer in agricultural fields.

This study evaluates the effectiveness of different sludge treatment technologies in reducing MP concentrations in sludge. The assessment involves analyzing sludge samples collected before and after treatment from five different WWTPs. The sludge treatments examined include thermal processes (incineration, pyrolysis), anaerobic digestion and mechanical dewatering processes.

The analysis of plastic polymers and their concentrations is conducted using Fourier-transform infrared spectroscopy (FTIR).

This study contributes to increasing knowledge regarding the management of MPs in wastewater treatment processes. By systematically evaluating the efficiency of sludge treatment technologies in MP removal, this research provides essential insights that can inform best practices for WWTP operations and the sustainable use of treated sludge in agriculture. The findings give important insight into the of selecting appropriate treatment methods at WWTPs to mitigate the environmental impacts of microplastics on terrestrial ecosystem and promote safer agricultural practices.

4.07.P-Mo352 Effect of Thermal Hydrolysis on the Behaviour of Target Organic Micropollutants in Sewage Sludge

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Growing concerns about organic micropollutants (OMPs) in sewage sludge (SS) are affecting its management strategy and disposal practices. Whilst, recovering nutrients and organic matter from digested SS for agricultural use is widely considered a positive contribution to the circular economy. One of the main concerns is that OMPs, such as pharmaceuticals, antibiotics and pesticides, may negatively affect environmental and human health when digested SS is used in agriculture.

Several pre-treatment technologies have been developed to stabilise and hygienise SS, to improve its dewatering and to facilitate its final disposal. Among these, the thermal hydrolysis process (THP) has proven effective in improving methane production in SS digestion, digestate dewaterability and pathogen removal. However, the fate of OMPs during and after THP is not yet fully understood. Some pollutants might degrade during the treatment, others might react with the present organic compounds.

This study investigates the behaviour of three target OMPs during THP, operated at temperatures of 160-200 °C, simulating the conditions in commercial THPs for sludge pre-treatment prior to anaerobic digestion. The persistence of the target OMPs will be assessed with analytical methods in both water with representative organic compounds and spiked SS samples, to understand the differences in the adsorption of different OMPs, characterised by comparable chemical properties. Moreover, the biochemical methane potential of the treated samples will be determined to assess the final biodegradation of the formed and/or residual compounds. We hypothesize that the OMPs will be better reduced after thermal treatment, because they will be (partially) degraded at the high temperatures, or they will be desorbed from the solid

sludge particles becoming available for biodegradation. Additionally, the desorbed compounds may react with other organic matter. The experimental results on the persistence of the thermally treated target compounds will be presented, along with findings from experiments using sewage sludge spiked with these three compounds and exposed to THP conditions.

By focusing on the potential interactions of the OMPs with recalcitrant organic compounds present in SS during the treatment, this research could pave the way for an improved (thermal) treatment process for SS that will also address the removal of OMPs and enhance the potential for direct reuse of the solid or liquid fraction.

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4.07.P-Mo353 Sorption and Biodegradation of Organic Micropollutants in Aerobic Granular Sludge

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Aerobic Granular Sludge (AGS) is a relatively new approach to wastewater treatment. As opposed to activated sludge with mainly flocculant biomass of relatively even composition, the granules in AGS differ in size and composition. Biomass ranges from flocs similar to activated sludge to granules of >6 mm. Granules also have distinct biomass due to the stratification of redox conditions across the granules. Typically organic micropollutants (OMPs) are removed via sorption and biodegradation during wastewater treatment, however this has yet to be thoroughly investigated for AGS systems. We hypothesized that different sized granules have potentially different sorption behaviour due to the differences in extracellular polymeric substances (EPS) and have different biodegradation patterns due to the differences in biomass composition among size fractions. Therefore, we investigated the capacity of AGS to remove OMPs via sorption and biodegradation by performing controlled batch experiments in the lab. We separated the sludge into different sizes in order to understand the role of different sludge composition on OMP removal.

We observed notable sorption (>40% removal) for 10 of the 24 OMPs tested in our study (Figure 1). The 10 OMPs include 3 fluoroquinolones (norfloxacin, ofloxacin, and ciprofloxacin), 3 macrolides (clarithromycin, azithromycin, and erythromycin), 2 beta-blockers (propranolol and atenolol), tetracycline, and citalopram. We noted that all of these 10 compounds are ionizable, with 6 positively charged compounds and 4 zwitterionic compounds at pH 7. Considering that sludge biomass typically has a negative charge, this seems to indicate electrostatic interactions between the 10 OMPs and the sludge. Larger fractions contributed more to sorption than smaller granules and flocs, as expressed per unit of biomass. Specifically, the normalized K_d for large fractions was up to 100% larger than for small fractions, suggesting that larger fractions contribute more to sorption in real AGS systems than smaller fractions and flocs. However, sorption kinetics were most likely retarded by diffusion limitations in large granules. Biodegradation was observed for a number of compounds. Overall, this study illuminated the roles of sorption and biodegradation in the removal of OMPs in AGS WWTPs, showing that these processes are size fraction dependent.

4.07.P-Mo354 Removal of Persistent, Mobile, Organic Compounds (PMOC) Using Activated Sludge-Based Hydrochars in a Decentralized Municipal Wastewater System

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Existing decentralized wastewater treatment systems already meet legal standards for water quality parameters (EU 2020/741). However, recent studies have demonstrated the uptake of persistent, mobile, organic compounds (PMOC) such as pharmaceuticals, tire additives and industrial chemicals by irrigated crops, raising concerns about human exposure through food consumption. The revised EU Urban Wastewater Treatment Directive (UWWTD 2024) outlines the need to include a quaternary treatment step that addresses micropollutants for safe water reuse in urban areas.

Hydrothermal carbonization (HTC) is a promising technology for the treatment of wet biological waste - it can recover nutrients and produce hydrochar, a carbon-rich solid residue with multiple applications. The aim of this study is to develop a sustainable sorbent from HTC of sewage sludge for the removal of PMOC from municipal wastewater. Sewage sludge, either alone or in combination with manure and seasonal biomass, is subjected to temperatures of 180-280°C and pressures of 10-65 bar to produce

hydrochar. The resulting hydrochar is activated by steam in a N₂ atmosphere at temperatures between 800 and 900°C for 1-4 hours to increase its carbonization and micro- and mesoporosity, which is beneficial for PMOC sorption. Preliminary results from sorption studies using 8 different activated sludge-based hydrochars show efficient removal of model PMOC perfluorobutanoic acid, perfluorooctanoic acid and sulfamethoxazole (logarithmic sorption coefficients K_d ranging between 3.3 to 5.4 for PMOC concentrations of 40 to 150 µg/L). The results also indicate that a de-functionalized, micro- and mesoporous sorbent performs best in terms of sorption of these compounds.

The best-performing activated hydrochar will be tested at an existing pilot site where wastewater from 8 population equivalents is treated by an aerated constructed wetland (CW). Water from the CW will pass through a slow trickling sand filter to reduce the bacterial load, followed by the reactor packed with the tailored activated hydrochar to remove residual PMOC. The final water quality from the decentralized treatment system is expected to be suitable for horticulture thereby improving water resilience in urban areas.

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4.07.P-Mo355 Biochar as a Green Remediation Approach for Per- and Polyfluoroalkyl Substances in Contaminated Stormwater

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Pyrolyzed organic matter, also known as biochar, has recently shown strong potential as a treatment solution for sorption of organic micropollutants in contaminated water. Biochar generally consists of a complex structure of carbonaceous fibers with different chemical composition and physical properties depending on the feeding material used for the pyrolysis. Due to its intrinsic hydrophobic characteristics as well as a variety of chemical moieties on its surface, biochar has been proved valuable for soil function improvement, by retaining nutrients and contaminants. In this study, we aim to evaluate the potential removal of per- and polyfluoroalkyl substances (PFAS) using biochar as a green remediation strategy for PFAS-polluted stormwater systems. To that end, different biochar materials (both commercial and research-based), as well as different feedstock (e.g. forest biomass, seed waste, sewage sludge) have been tested. At first, static flow systems were carried out over 7 days in a set of 12 different biochar materials to screen for the best performing ones as a pre-selection for the subsequent experiments. As the next step, 5 selected materials were used for filter tests in a constant-flow column experimental set-up. With the final goal of identifying retention rates as well as breakthrough levels for the 15 PFAS tested, the columns were constantly running over a period of 69 days. Our observations indicated that there were large differences in the biochar performance when it comes to the retention rates of the tested PFAS contaminants. Some materials, either plant- or sludge-based, showed relevant capacity to remediate PFAS from polluted stormwater in the long term, while others showed a poor performance to adsorb PFAS. Thus, biochar performance for removal of PFAS depends, among others, on both the feeding material and production conditions. Overall, static flow tests showed a very promising application of biochar as a remediation tool for PFAS with some materials reaching removal efficiencies up to 99% in static systems. Also, long-chain PFAS showed a stronger sorption to the biochar compared to short-chain PFAS. For the dynamic flow systems, similar observations were achieved. Short-chain PFAS tended to remain in the aqueous phase and, thus, column breakthrough occurred at an earlier stage. However, overall biochar has been proven as a promising and green strategy for the removal of PFAS from polluted stormwater.

4.07.P-Mo356 Understanding Sorption and Enhancing Sorption of OMPs in Soils to Optimize Nature-Based OMP Removal in Drinking Water Treatment

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Organic micropollutants (OMPs) at trace level in surface waters can threaten sources for drinking waters. Soil passage is a commonly used nature-based pre-treatment of surface water used in drinking water production. Previous studies have shown that certain OMPs can be removed during soil passage, whilst other OMPs are not removed. Also, removal efficiencies can vary greatly between different locations. Sorption to soil is hypothesized to be an important OMP removal mechanism. Especially for OMPs that are ionizable at environmentally relevant pH, sorption behavior is not well understood. Also, the lack of reliable experimental data hinders the development of reliable predictive models for OMP sorption.

We here present insights from sorption tests that were carried out according to the OECD-106 guideline on sorption-desorption batch experiments with different soils and over 60 OMPs (including pharmaceuticals, pesticides, industrial chemicals, PFAS) that were selected based on their occurrence in Dutch surface waters. Three different soils - cyclone sand, aquifer material from 7-8m depth and infiltration pond material from 0-1m depth were tested for their OMP sorption. The cyclone sand was a washed sand acting as a blank sandy soil, whereas the aquifer and infiltration pond sediment represented Dutch drinking water aquifers and soil passage, respectively. Secondly, green sorbent amendments biochar, peat, and iron sludge from rapid sand filters - were added to these three soils to explore how sorption of mobile OMPs could be enhanced in the soil passage. These sorbents were tested to identify the most suitable material for field application, balancing practical considerations and enhanced sorption. In summary, this study focused on understanding and enhancing the sorption of OMPs to different soils and soil amendments with special attention to ionizable OMPs. The results created a better understanding of OMP sorption to soils - especially charged OMPs- and served as input for the design of lab-scale soil passage systems optimized for OMP removal.

4.07.P-Mo357 Photochemical Degradation of a Mixture of Pharmaceuticals in Wastewater

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Organic micropollutants, such as pharmaceuticals, represent a significant environmental and public health challenge due to their persistence, toxicity, and potential to contribute to antimicrobial resistance. These contaminants often pass through conventional wastewater treatment, entering aquatic environments and posing risks to ecosystems and human health. Advanced oxidation processes (AOPs), particularly photoelectrochemical oxidation (PEC), offer a promising solution for removing trace-level pharmaceuticals from secondary-treated wastewater. However, the photocurrent conversion efficiency, catalytic activity, and stability of BiVO₄-based photoanodes, which are key components in PEC systems, are still limited. Surface modifications have shown potential to address these challenges and enhance their performance. This study aims to improve the PEC activity of BiVO₄-based photoanodes for the degradation of pharmaceutical mixtures in secondary-treated wastewater through surface modification using quaternary ammonium-based compounds (QACs). The photoanodes were fabricated using ultrasonic spray pyrolysis and characterized using techniques such as scanning electron microscopy (SEM), X-ray diffraction (XRD), and linear sweep voltammetry (LSV). PEC experiments were conducted under simulated solar light to assess the degradation of a mixture of pharmaceuticals at an initial concentration of 10 µg/L.

The results demonstrated that the surface modification of BiVO₄ with QACs significantly enhanced the degradation rate of pharmaceuticals compared to unmodified BiVO₄ photoanodes. SEM images confirmed the successful deposition of needle-like QAC particles on the BiVO₄ surface, leading to improved charge separation. Notably, pharmaceuticals such as diclofenac, sulfamethoxazole, sulfadimethoxine, and acetaminophen showed higher removal rates in the presence of the modified photoanodes. This research highlights the potential of QAC-modified BiVO₄ photoanodes as an effective approach for enhancing the degradation of pharmaceuticals in wastewater. The findings contribute to advancing the field of PEC-based wastewater treatment technologies and offer promising implications for upscaling and practical application in treating pharmaceutical-contaminated wastewater.

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4.07.P-Mo358 Treatment of Pharmaceutical Industry Wastewater Using Peroxydisulfate Activated by Nano-Scale Zero Valent Iron

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Wastewaters from the pharmaceutical manufacturing contain high concentrations of active pharmaceutical ingredients (APIs) that are resistant to conventional wastewater treatment methods, posing significant environmental risks. Traditional approaches like thermal oxidation and activated carbon sorption face challenges as thermal oxidation is energy-intensive and activated carbon sorption generates secondary waste without addressing degradation. Advanced oxidation processes (AOPs) offer a promising

alternative, with peroxydisulfate (PDS) showing strong potential due to its ability to produce reactive sulfate radicals and other reactive oxygen species. Despite this, its application in API degradation remains relatively under-investigated, with notable gaps, particularly in the context of pharmaceutical wastewaters. This study examines the degradation of the synthetic azasteroid Finasteride in wastewater from its production, using PDS activated by nanoscale sulfidized zero-valent iron (nZVI). Key operational parameters, including temperature, pH, and the presence of scavengers and surfactants, were systematically evaluated. Response surface methodology (RSM) was employed to optimize the system by analysing interactions between variables and their effects on degradation efficiency. Optimal conditions were achieved with an equimolar nZVI-to-PDS ratio and low carbonate concentrations. Interestingly, a molar surplus of nZVI-to-PDS diminished degradation efficiency, revealing a non-linear relationship. Neither initial pH nor chloride concentration significantly affected the process. These results indicate the potential of PDS/nZVI systems as environmentally sustainable solutions for pharmaceutical wastewater treatment. The findings also highlight the value of RSM in designing and optimizing advanced treatment systems for complex wastewater matrices.

4.07.P-Mo359 Removal of Perfluorooctanoic Acid Using Biopolymer Functionalized Graphene Oxide Nanocomposites: Isotherm, Kinetics, and Thermodynamics

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Perfluorooctanoic acid (PFOA) is a recalcitrant emerging organic contaminant from the per- and polyfluoroalkyl substances (PFAS) family that poses serious environmental challenges. PFOA has been extensively produced and utilized across various industries, such as textile and paper coatings, metal plating, semiconductor manufacturing, etc. Its widespread occurrence and significant bioaccumulation have sparked growing global concern, underscoring the urgent need for effective removal strategies. Among existing treatment methods, adsorption has shown considerable effectiveness in removing PFOA, and there is a continued need to develop more efficient adsorbents for this purpose. In this context, this study prepared ultrasonic-assisted chitosan-functionalized graphene oxide (GO-CS) nanocomposites and used it for the removal of PFOA. The surface characteristics of the nanocomposites are tailored by adjusting the chitosan loading amounts to attain optimal performance in PFOA adsorption. Different advanced characterization results confirmed the successful functionalization of graphene oxide with chitosan. In batch experiments, the most effective GO-CS nanocomposite achieved approximately 95% PFOA removal under favorable conditions: an adsorbent dose of 240 mg/L, pH 4, and a contact time of 120 min. Non-linear regression analysis revealed that the adsorption process followed pseudo-second-order kinetics and was best represented by the Langmuir isotherm model, with a maximum adsorption capacity of approximately 642 mg/g. The thermodynamic analysis revealed that the adsorption of PFOA onto the developed adsorbent was spontaneous and exothermic in nature. Spectroscopic and zeta potential analyses confirmed that electrostatic interaction and hydrogen bonding are the primary mechanisms facilitating PFOA adsorption. The regeneration study demonstrated that the developed adsorbent could be reused across multiple regeneration cycles while maintaining significant adsorption performance. This study provides valuable insights into the adsorption behavior of PFOA and highlights the substantial potential of chitosan-functionalized graphene oxide nanocomposites as highly efficient adsorbents for PFOA removal.

4.07.P-Mo360 Preparation of Green Synthesized Nano-Catalyst for the Sono-Catalytic Degradation of Emerging Organic Contaminants

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Emerging Contaminants (ECs) are group of chemical substance that are resistive to degradation and recently have been detected in surface waterways, groundwater, drinking water, food sources, and municipal wastewater. Trace amounts of ECs such as per-fluorinated compounds (PFCs), per fluorinated alkyl substances (PFAS), perfluorooctanoic acid (PFOA), endocrine disrupting compounds, pharmaceuticals, synthetic dyes, phenolic compounds, microplastics and alkyl benzene sulfonates have been found in most terrestrial and aqueous ecosystem. Due to this, global attention has been drawn to the transport, retention, bioaccumulation, and adverse consequences of ECs that are toxic to human health even at lower concentration. This study aims to improve the degradation rate of ECs from wastewater by using green-synthesized nano-catalyst prepared from lantana camara plant leaf extract under ultrasound radiation. Nanoparticles' distinct physicochemical characteristics allow them to have a significant capacity for adsorption and catalytic degradation of organic compounds. The use of ecologically friendly synthesis methods for nanoparticles is consistent with the increased focus on sustainable and green technologies.

Sono-catalytic study is conducted using an ultrasound reactor to determine the degradation rate of ECs in the presence of nano-catalyst. The green synthesized nanomaterial was prepared by leaf extract from *lantana camara* with titanium dioxide (TiO₂) powder for its application in ECs degradation. Post synthesis, the catalyst was characterized in order to determine its structural characteristics, such as particle size and composition using scanning electron microscope (SEM), transmission electron microscopy (TEM), Energy Dispersive X-Ray (EDX), and Fourier-transform infrared spectroscopy (FTIR). The formation of tetragonal-shaped TiO₂ nanoparticles was validated by a variety of characterisation techniques.

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4.07.P-Mo361 Evaluation of the Environmental Toxicity of Wastewater Treated by the Emerging Solar Chlor-Photo-Fenton Process for Reuse in Agricultural Irrigation

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The toxicity of wastewater treated by applying the novel solar chlor-photo-Fenton (SCPF) process has been assessed for the first time. This innovative process, based on the simultaneous supply of hydrogen peroxide (H₂O₂) and sodium hypochlorite (NaOCl) using ferric nitrilotriacetate (Fe³⁺-NTA) as an iron source, has shown promising results in terms of disinfection and removal of organic microcontaminants while minimizing disinfection by-products occurrence.

In the present study, secondary effluents from wastewater treatment plants (WWTPs) in Uleila del Campo and El Toyo (Almería, Spain) were treated using the SCPF process with low reagent concentrations: 0.1 mM of Fe³⁺-NTA (molar ratio 1:1, 5.5 mg/L Fe³⁺), 0.73 mM of H₂O₂ (25 mg/L), at different chlorine doses ranging from 0.13 mM (10 mg/L) to 0.39 mM (30 mg/L). Conventional chlorination at comparable chlorine concentrations and solar photo-Fenton were also evaluated in parallel to compare with the novel strategy.

The potential environmental toxicity of the treated wastewater was evaluated using different bioassays. Microbial toxicity was evaluated using the luminescent bacteria test with *Aliivibrio fischeri*, and a new microrespirometry toxicity test targeting responses of microplankton communities. Phytotoxicity was evaluated using a freshwater algal growth inhibition test with the unicellular green algae *Raphidocelis subcapitata*, and a germination test using emergence of lettuce seedlings (*Lactuca sativa* L.). The genotoxicity of treated wastewater was evaluated using the Umu-C and the Ames fluctuation tests. The traditional bioluminescence inhibition test showed no apparent toxicity regardless of the wastewater treatment process. In contrast, the freshwater algal test showed detectable phytotoxicity in wastewater treated by conventional chlorination, while no or very low phytotoxicity was detected in samples treated with the other processes. The lettuce germination test indicated low phytotoxicity for a few undiluted samples but could be mitigated by dilution. No or very low apparent genotoxicity was detected for the treated wastewater samples. Overall, comparable or lower ecotoxicity and genotoxicity were observed for wastewater treated with SCPF compared to conventional processes. This research sheds light on the application potential of the novel SCPF process and may pave the way for large-scale sustainable applications.

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4.07.P-Mo362 Phytotoxicity as a Tool for Evaluating Toxin Removal from Industrial Waste Following EGSB Reactor Operation

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The wet coffee bean processing generates two main wastes: solid lignocellulosic material (husk and pulp) and wastewater. With Brazil producing 3.6 million tons of coffee annually, this results in 27 to 432 million

tons of solid waste and 1.8×10^3 to 5.4×10^3 liters of wastewater. Coffee waste is rich in carbohydrates and organic matter but contains toxic polyphenols, leading to environmental issues like aquatic eutrophication if improperly disposed of. Anaerobic digestion presents a promising solution by reducing organic matter and producing bioenergy (CH_4). Combining coffee residues with brewery wastewater can enhance biogas production, as polyphenols are diluted, improving organic matter utilization. Brewery wastewater production is substantial, with 10 30 m³ of water used per ton of beer, yielding 10 20 m³ of wastewater [3]. Expanded Granular Sludge Bed (EGSB) reactors are effective for wastewater treatment, enhancing COD removal through bed expansion, improving organic matter reduction. To confirm the efficiency of anaerobic digestion in reducing toxicity, phytotoxicity studies provide a reliable assessment tool. This study investigated the effluent from an EGSB reactor using phytotoxicity assays to evaluate its efficiency in treating the combined wastes.

4.07.P-Mo363 Assessing Ecotoxicity of Textile Effluents: A Comparative Study of Electrochemical Ozone Oxidation (ECOOP) and Conventional Treatments with Focus on River Ecosystem Impact

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The textile industry is one of the significant water-intensive industries in the world that generates 90% of the consumed water as wastewater. Generally, the wastewater discharged from the textile industry contains dyes, heavy metals, scattering materials, acids, alkalis, salts, and detergents. Among these contaminants, it is becoming particularly a challenging problem to degrade dyes due to their complexity. Incomplete removal of these pollutants during conventional treatment processes may result in the discharge of harmful byproducts into the water bodies. This can have detrimental effects on aquatic ecosystems, disrupt food chains, and deteriorate water quality, ultimately causing ecological imbalances. To address the environmental impacts associated with conventional treatment technologies, an electrochemical-based advanced oxidation process called the Electrochemical Ozone oxidation process (ECOOP) was assessed for its efficacy in the complete mineralization of textile effluent. The primary objective was to tailor the treatment process to minimize the formation of undesirable toxic byproducts, making it suitable for safe discharge. Laboratory-scale studies demonstrated that the proposed treatment system could achieve a remarkable 65% removal of total organic carbon (TOC) from real textile effluent. Implementing this system in textile wastewater treatment will have the added benefit of eliminating the need for chlorination, consequently reducing the formation of chlorinated disinfection byproducts. Furthermore, it is crucial to study the potential impacts of contaminants on ecosystems and aquatic organisms. Relying solely on chemical analysis may not provide a complete picture of the toxicity levels of treated wastewater. Therefore, there is a need to develop holistic and impact-oriented methods to assess the ecotoxicity of textile effluents. In this study, ecotoxicological analysis was conducted on samples treated with ECOOP and conventionally treated samples from a CETP. Results revealed differences in toxicity levels, providing valuable insights for assessing the environmental impact of this treatment technology.

4.07.P-Mo364 Spatial Distribution of Pharmaceutical Compounds and Pesticides Concentration in Zarqa River, Jordan

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The Zarqa River is critical to Jordan's agricultural supply and social development. It is used to irrigate a variety of crops, including vegetables, fodder crops, trees, and commercial crops. The river is facing tremendous challenges caused by contamination from various sources, including industrial and domestic wastewater overflow. Several WWTPs release treated effluent into the Zarqa River. In addition to these effluents, another significant source of contamination arises from the unregulated consumption of PhACs. Furthermore, industrial discharges from pharmaceutical, pesticide, and fertilizer manufacturing plants, coupled with the improper disposal of expired or unused medications, represent pollutants of concern. These contaminants pose serious risks to aquatic ecosystems in the Zarqa River (ZR) and beyond, impacting both environmental and human health. Therefore, understanding the spatial distribution of these pollutants in ZR is critical for effective environmental management, public health protection, and safe

reuse practices in irrigated agriculture in Jordan Valley.

The study focuses on Assamrah WWTP's great efficiency in removing major physical and chemical water quality factors, with effluent matching Jordanian regulations (JS893/2021) for discharge and agricultural irrigation. The water quality of the Zarqa River and other sample sites was declared appropriate for irrigation of industrial and field crops, as well as forest trees. However, pharmaceutical residues, particularly carbamazepine, showed persistence, with concentrations increasing downstream indicating resistance to breakdown. Ofloxacin and diclofenac were greatly reduced, although pesticide residues such as chlorpyrifos and diazinon had high influent levels that significantly decreased downstream, suggesting effective WWTP treatment and natural dilution. Despite the reductions, stable chemicals such as diuron remained detectable. The absence of THMs demonstrates good management at WWTP.

These findings highlight the widespread prevalence of pharmaceutical and pesticide residues in environmental matrices, necessitating improved treatment technologies, public awareness campaigns, regulatory enforcement, and continued monitoring to reduce their environmental and health effects. The study establishes a vital baseline for regulating OMPs and optimising wastewater treatment systems.

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4.07.P-Mo365 Evaluating Organophosphate Flame Retardant (OPFR) Removal in Drinking Water Treatment: A Multi-Site Study in England and Wales

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Flame retardants are functional additives designed to prevent ignition or slow fire progression. These chemically diverse substances are crucial for enhancing the fire safety of various products used both in industry and daily life. Organophosphate flame retardants (OPFRs) are commonly used as additives in items such as building materials, electrical devices, and upholstered furniture. Since OPFRs are not chemically bonded to these materials, they can be easily released into the environment, resulting in their widespread detection in both environmental and biological samples. Concerns about OPFRs in drinking water are growing due to their potential health risks, including neurotoxicity, cancer, and endocrine disruption. However, information on OPFR levels in drinking water and the efficacy of current treatment processes in removing these chemicals remains limited.

This study aimed to examine the presence of OPFRs in untreated and treated drinking water and to assess how effectively current treatment processes remove these contaminants at facilities across England and Wales. A risk-based approach was utilised to select three drinking water treatment plants with various water sources and treatment methodologies. Samples were collected at three stages in the treatment process: raw water, intermediate treatment stages, and final drinking water at four intervals throughout 2024. A range of OPFRs were analysed in these samples to better understand their presence and the likelihood of their removal during water treatment. The findings that will be presented from this monitoring program provide insights into the performance of advanced and conventional treatment processes.

The results of this study will inform further monitoring actions to ensure safe water supplies, support the creation of guidance for water companies, and aid in shaping water safety plans. Additionally, the findings will be valuable for future UK policy development concerning these emerging contaminants of concern.

4.07.P-Mo366 Removal of Micropollutants in Phytoparking Pilot: Insights from the MULTISOURCE Project

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The EU Horizon 2020 MULTISOURCE project promotes the use of nature-based solutions (NBS) for urban wastewater treatment and reuse across seven diverse pilot sites in Europe and the USA. These pilots showcase Enhanced Natural Treatment Systems (ENTS) designed to improve water quality, promote sustainable reuse, and boost urban resilience to climate change. This study focuses on the Phytoparking pilot in Belgium, an innovative 76 m² dual-stage aerated hybrid wetland implemented at an off-grid campsite. The system efficiently separates and treats grey and black wastewater streams at their source. Over two years of continuous monitoring during the active seasons, the Phytoparking system

demonstrated high performance, achieving >90% removal of many organic micropollutants, including challenging compounds such as carbamazepine (with >50% reduction) and oxazepam (with >30% reduction). It also achieved significant pathogen removal, consistently meeting local discharge standards for BOD, COD, TSS, and pH, as well as Class C criteria under the European Irrigation Regulation EU2020/741 for water reuse.

The Phytoparking pilot's robust operation underscores the potential of NBS to provide effective wastewater treatment solutions that can be integrated into multifunctional urban spaces, such as parking areas. Its success showcases the feasibility of adopting NBS for wider urban applications to enhance water resilience, conserve water resources, and comply with the recently adopted EU Wastewater Treatment Directive. The findings from the Phytoparking system contribute valuable insights into how nature-based solutions can be scaled and integrated into urban and peri-urban areas across Europe and beyond, aligning with global sustainability efforts to mitigate water scarcity and improve ecological resilience while providing economic and societal benefits.

4.07.P-Mo367 Novel Laser Pre-Structured Silicon Filters for Micro- and Nanoplastics Analysis

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Microplastics defined according to DIN/TR 21960:2020 as plastic particles in the size range of 0.001 to 1 mm have become a pervasive and concerning pollutant in aquatic environments worldwide. It originates from a variety of sources, including plastic debris fragmentation, microbead abrasion, and synthetic fiber shedding, and these minute particles pose significant threats to marine ecosystems, wildlife, and human health. Despite growing awareness of the issue, effective methods for their detection and removal from environments remain limited, highlighting the urgent need for innovative solutions. The ever-decreasing size of microplastic particles, with some reaching as small as nanometers, presents a formidable challenge for existing technologies to identify and remove them from the environment accurately. Silicon filters offer a promising solution for filtering out microplastics, because they do not introduce contamination, maintaining the purity of samples during filtration processes. Additionally, silicon exhibits a Raman shift that minimally overlaps with the Raman spectra of common microplastics. This spectral separation facilitates clearer detection and identification of microplastics in Raman spectroscopy analyses, as it minimizes background interference from the filter material itself. However, the development and fabrication of such filters are costly and complex, primarily due to the reliance on photolithographic mask processes required to achieve the desired pore geometries.

For this purpose, our study focuses on an innovative laser-based approach to streamline the fabrication of silicon filters with precise pore geometries. The effectiveness of this method is demonstrated by the successful fabrication of a silicon filter with elongated 15-micrometer pores. Moreover, this study also explores a novel cascade filtration system incorporating elongated 15-micron holes and a 90 nm aluminum oxide (AlOx) filter. The design combines high throughput with precision, targeting microplastics across a broad size range. The elongated holes ensure effective pre-filtration by capturing larger particles, while the AlOx filters enhance selectivity for submicron debris. This approach offers a scalable solution for water treatment applications, advancing microplastic removal technologies.

4.07.P-Mo368 Co-Benefit or Antagonisms of a Reed Filter for Water Treatment and Biodiversity?

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As part of the treatment of run-off water from the Paris western ring road, the City of Paris has installed an extensive filtration system in the Bois de Boulogne site (West of Paris woods). The aim of the system is to combine the need to clean up polluted water with the need to preserve biodiversity.

In addition to treating polluted water, particularly in terms of Trace Metal Elements (TME) and polycyclic aromatic hydrocarbons (PAH), our objective is to study the colonisation dynamics of the sandy substrate of the reed filter and the influence of changes in physico-chemical parameters on the filter ecology, and to verify the impact of treated water discharges on the receiving aquatic environment. For 2 years, we monitored changes in substrate properties (pH, organic matter, cation exchange capacity, etc.), levels of

TME and PAH contamination, enzymatic activities in the environment, and earthworm and enchytraeid populations (density, specific richness). At the same time, we caged gammarids, a model aquatic invertebrate, in the receiving river, upstream and downstream of the planted filter discharge, and measured sublethal biomarkers to characterise the impact of the filtration system on the receiving environment. As the filter plant evolved, and in particular as the sedimentation and reeds enriched the substrate with organic matter, an increase in the oligochaetes density and diversity was observed. The results show that the dynamics of planted filter colonisation are strongly linked to changes in the properties of the environment, in particular the level of organic matter. Although contamination levels are increasing, they remain relatively low and have no impact on the biological colonisation dynamics of the filter. On the other hand, discharges of treated water are beneficial to the receiving environment, firstly because contaminant levels are fairly low, and secondly because the treated water helps to improve some river parameters, particularly oxygen level. The planted filter system is therefore a water treatment system of ecological interest.

In the future, the long term monitoring of the chemical, physico-chemical and ecological dynamics in the filter will enable to assess the benefits or limitations of this type of water treatment system in terms of environmental quality and biodiversity.

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4.07.P-Mo369 Simultaneous Partial Nitritation, Anammox and Denitrification Reactor: A Focus on Alkalinity Availability Under Mainstream Conditions

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Simultaneous partial nitritation, anammox, and denitrification (SNAD) in a single-stage reactor has emerged as a feasible and sustainable approach for mainstream nitrogen removal. SNAD offers several advantages over conventional biological nitrogen removal (CBNR), such as reduced costs associated with aeration, sludge generation, and disposal. However, maintaining long-term stable SNAD operation remains challenging due to the simultaneous growth of different functional microorganisms. Municipal wastewater typically contains nitrogen concentrations of 1.8-4.3 mEq N L⁻¹ and alkalinity levels between 1.5-4.0 mEq CaCO₃ L⁻¹. While CBNR processes generally consume alkalinity at a rate of 1.0 mEq CaCO₃ per mEq N removed, the role of alkalinity in SNAD processes is still not fully understood. This study aimed to evaluate alkalinity availability in a SNAD reactor fed with anaerobically pre-treated municipal wastewater (APMW). The bioreactor was operated over 90 days in three experimental phases: (I) SNAD start-up and granular biomass adaptation from synthetic wastewater to APMW; (II) addition of biocarriers to improve biomass retention; and (III) an increase in the hydraulic retention time from 8 to 12 hours. During phases I and II, ammonium removal rates reached approximately 46% and 61%, respectively, which primarily drove alkalinity consumption in the reactor by nitritation and anammox reaction. Additionally, denitrification likely contributed to alkalinity production, resulting in increased effluent concentrations of 9.5 mEq L⁻¹ and 9.7 mEq L⁻¹, respectively. In phase III, high and stable performance was observed for both organic matter (75% COD) and nitrogen (70% N) removal, along with alkalinity production of approximately 1.5 mEq L⁻¹, or 0.8 mEq CaCO₃ per mEq N removed. This production rate helped regulate effluent pH, which ranged from 7.4 to 7.6, ensuring suitability for wastewater discharge in water bodies. Moreover, optimal mEq HCO₃⁻/mEq NH₄⁺-N ratios reported for partial nitritation and anammox processes range from 0.2-0.9 and 1.2-3.5, respectively; in this study, stable SNAD performance was achieved at an approximate ratio of 3.7 mEq HCO₃⁻/mEq NH₄⁺-N. Overall, these findings demonstrate that using APMW in a SNAD reactor provides adequate influent alkalinity for bioprocessing and results in suitable effluent alkalinity for subsequent wastewater disposal.

4.07.P-Mo370 Aqueous Phase Mitigation of Chemical Oxygen Demand in Wastewater using Adsorbents from *Delonix regia* Pod Husks

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The present study aimed to compare the adsorption efficiency of an adsorbent derived from agricultural waste, specifically *Delonix regia* pod husks, as the widespread use of commercial activated carbon is often limited by its high cost. The *Delonix regia* pod husks were ground into a fine powder using a mortar and

pestle. This powdered material was then sieved using a 0.25 mm mesh sieve and steeped overnight in a saturated ammonium chloride solution (NH_4Cl) for chemical activation. The resulting slurry was filtered and the filtrate was discarded. The residue was thoroughly washed with distilled water and subsequently sun-dried. Next, the activated material was divided into two portions. The first portion produced the chemically activated adsorbent, designated as ADR, while the second portion underwent pyrolysis in a muffle furnace (NEY M-525) at 350°C for 1.5 hours to yield the activated carbon, referred to as CDR. Both adsorbents, ADR and CDR, were utilized to treat wastewater, focusing on the removal of organic components responsible for the chemical oxygen demand (COD) in industrial wastewater. The adsorption process was assessed through batch adsorption experiments, measuring the amount of COD adsorbed (mg.g^{-1}). Various factors were investigated, including adsorbent dosage, pH, contact time, and temperature. The optimal adsorbent dose was determined to be 2.5 g for both ADR and CDR, achieving removal rates of 64.21% and 66.48%, respectively. FTIR analysis indicated absorbance peaks at 2363 cm^{-1} and 2963 cm^{-1} for OH functional groups, and at 3226 cm^{-1} for NH_2 groups, suggesting that the adsorbent CDR has a higher adsorption capacity than ADR. The equilibrium data for COD removal were found to fit both the Linearized Langmuir and Freundlich models well. This strong correlation between the experimental adsorption rate data indicates that these models could be effectively applied in design applications.

Keywords: Activated Delonix regia (ADR), Carbonized Delonix regia (CDR), Chemical Oxygen Demand (COD), Potential of Hydrogen (pH), Fourier Transform Infrared Spectroscopy (FTIR)

4.07.P-Mo371 Removing Chromium (VI) from Wastewater with Crosslinked Calciumphosphate/Chitosan/Gelatine Coated Polylactic Acid Pellets

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Chromium (VI) [Cr(VI)] is, among others, toxic and carcinogenic. Nevertheless Cr(VI) compounds are still used in different industries such as tanning and chromium plating and can be found in soil and water around the factories in various concentrations. Chitosan/crosslinked chitosan can be used for the removal of Cr(VI) from wastewater. However the synthesis of crosslinked chitosan is often time consuming. In addition the data situation in the analytical field have so far been unsatisfactory, especially in regards to the possible reduction of Cr(VI) to Cr(III) . However this information is crucial because Cr(III) is less dangerous than Cr(VI) .

The current work is an extension to existing systems and focuses on calciumphosphate/crosslinked biopolymer-coated poly(lactic acid) pellets for the removal of chromate from aqueous solution, where the biopolymer is chitosan or gelatine. The best adsorption results were obtained by a crosslinked chitosan/gelatine material (99.6% removal in 24 h). All three materials were analyzed by scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), X-ray photoelectron spectroscopy (XPS), UV-VIS spectroscopy, X-ray microprobe and inductively coupled plasma optical emission spectroscopy (ICP-OES). Microprobe and ICP-OES measurements point out the successful adsorption of chromium. A color change of all three materials to a greenish tone suggested the reduction to Cr(III) and XPS and UV-VIS spectroscopy prove that Cr(VI) can be reduced by the material. Overall, the data suggest a possible application of chitosan materials in wastewater treatment where adsorption and Cr(VI) to Cr(III) reduction can be combined in one material.

All in all the results show that the addition of chitosan is crucial for the adsorption of Cr(VI) . By adding gelatine the adhesion of the coating can be improved. Current challenges include upscaling of the synthesis and adsorption experiments, using real wastewater and substitution of glutaraldehyde by a biocompatible and biodegradable crosslinker.

4.07.P-Mo372 Mitigation of Hydrogen Sulfide Emissions and Corrosion in Wastewater Treatment Using Ferric Chloride Foam: A Comparative Study with FeCl_3 Mixing

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Hydrogen sulfide (H_2S) emissions from wastewater sludge cause intense odors and severe corrosion in treatment facilities. While ferric chloride (FeCl_3) mixing is a common strategy for reducing H_2S emissions, its efficiency decreases at high concentrations. This study investigates the use of ferric chloride foam (FC-foam) as an alternative surface application method and compares its effectiveness with traditional FeCl_3 -sludge mixing.

Batch tests revealed that FC-foam reduced cumulative H_2S emissions by 86.44%, significantly outperforming the 49.22% reduction achieved by FeCl_3 mixing. In semi-continuous tests, FC-foam

application reduced H₂S concentrations by 91.40% (from 5125 ppm to 440.5 ppm), compared to a 71.38% reduction (from 5125 ppm to 1466.5 ppm) with FeCl₃ mixing. FC-foam mitigates H₂S emissions through two primary mechanisms: forming a stable 28-hour barrier that prevents H₂S release and leaching ferric chloride to react with sulfide ions on the sludge surface.

The study also evaluated the biodegradability and corrosion-inhibition properties of the AOS surfactant in FC-foam. Biodegradability tests confirmed near-complete degradation within 10 days, while corrosion tests demonstrated a 61.19% to 67.04% reduction in corrosion on carbon steel compared to FeCl₃ mixing. These findings suggest that FC-foam is a promising alternative for maintaining low H₂S concentrations (6-8 ppm) and mitigating corrosion, offering a simple yet effective solution that addresses the limitations of conventional FeCl₃ mixing in wastewater treatment.

4.07.P-Mo373 Effluent Reuse in Greenhouse Horticulture: Measures to Ensure Safe and Sufficient Irrigation Water

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Due to changing rainfall patterns, water supply in greenhouse horticulture is under pressure. While most growers currently rely on rainwater as the primary source for irrigation, this is generally insufficient to meet the needs of plants. As a result, growers often use supplementary sources, such as groundwater, which is a precious resource which faces future restrictions. Effluent from wastewater treatment plants, which can be treated to become suitable for irrigation, is increasingly considered as an irrigation source. However, growers are hesitant to adopt this solution without guarantee that the effluent is safe for human, animal, and plant health and the environment. Here, measures are presented to provide water with the required quality requirements to guarantee safe irrigation for the crops, employees, and consumers of the crops. They are included in a framework that entails water treatment, process monitoring, water quality monitoring and risk assessment.

As effluent quality can fluctuate over time it is important to implement treatment processes such as reverse osmosis combined with advanced oxidation. This treatment can remove many contaminants, although some may remain. A risk analysis, including a quantitative microbial risk assessment (QMRA), is required to meet EU regulations and address health risks. Findings suggest that additional treatment steps, such as ultraviolet (UV) or ozone disinfection, are necessary to ensure safe water for irrigation. Moreover, the current EU regulations do not address risks to plants, which are a significant concern for growers. While treatment processes like reverse osmosis and UV can reduce microbial contaminants, the acceptable levels of phytopathogens and their treatment remain unclear. As part of the chemical and ecological risk assessments, the use of bioassays is explored for testing the effects of specific contaminants on plant growth.

In conclusion, regulations and trade practices allow the use of treated effluent as an irrigation water source, and advanced treatment processes ensure a water quality that can mitigate microbial and chemical risks. Monitoring and verification systems are essential to ensure the continued safety and efficacy of effluent as irrigation water. In this way, safe effluent reuse in greenhouse horticulture can contribute to sustainable water use.

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4.08 Antimicrobials and Antimicrobial Resistance in the Environment

4.08.T-01 Minimum Selective Concentrations of Antibiotics: A Resistance Cost-Based Approach Towards Representative Estimates

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Selection for antibiotic resistance in bacteria is known to occur at drug concentrations far below therapeutic levels. At the same time, reports of elevated concentrations of antibiotics in contaminated

environments call for the prediction of no effect concentrations (PNECs) and corresponding regulatory measures. The standard approach to the derivation of PNECs addressing selection for antibiotic resistance is centered around the minimum selective concentration (MSC). The latter is defined as the drug concentration where resistant and susceptible bacteria are subject to equal fitness. Competition assays provide straightforward means to determine the MSC for particular pairs of resistant and susceptible strains. However, in light of substantial inter- and intra-species diversity, the large number of resistance gene variants, their embedding in alternative genetic contexts, and the variability of environmental conditions, it is hardly feasible to derive truly representative MSC values through experiments. Consequently, attempts have been made to derive minimum values of the MSC from existing data for subsequent use as PNECs. The most prominent approach computes the PNEC as the lowest observed inhibitory concentration (lowest MIC) of any bacterial strain multiplied by an assessment factor of 1/10. Here, we demonstrate that the factor providing the link between MSC and MIC is equivalent to the cost of resistance. Specifically, the cost reflects a relative drop in the intrinsic growth rate associated with the resistance trait. Because the distribution of resistance costs can be estimated from existing data with reasonable accuracy, we propose to employ this information in the derivation of PNECs. Our results suggest that the current, most widely used approach ($\text{PNEC} = \text{lowest MIC} * 1/10$) is rather tolerant. In this contribution, we provide arguments for a more stringent approach considering, e.g., the very low costs of laterally transferable plasmid-borne resistance or the yet underexplored consequences of evolutionary cost reduction.

4.08.T-02 Antibiotics Residues in Irish Sediments

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This study assesses the concentrations of a range of antibiotics in riverine and transitional sediments in Ireland. A selection of 12 macrolide, fluoroquinolone, sulphonamide, and diaminopyrimidine antibiotics were quantified in 80 grab surficial sediment samples from around Ireland, selected to investigate areas of potentially higher pollution risk (agriculture, aquaculture, industrial emissions, and wastewater emission points) as well as isolated areas where there are no known pollution sources. Several of the macrolides and sulphonamides/trimethoprim were generally detected more frequently above limits of quantification (LoQ). Fluoroquinolones, while frequently detected above limits of detection (LoD), concentrations were mostly below method LOQs. The most prevalent antibiotic detected was clarithromycin, found at the highest mean concentration (6.65 ng/g) and detected in ~90 % of samples. Comparing levels of quantified antibiotics to levels reported internationally, Ireland is at the lower end for all quantified antibiotics. This is with the notable exception of clarithromycin, which is higher than levels found in comparable studies in Italy, Spain, France, and Argentina. Higher levels of total antibiotics (49.3 ± 24.7 ng/g) were found to be present immediately adjacent to wastewater emission points while moderate degrees of contamination (9.0 ± 9.7 ng/g) were also linked to wastewater, aquaculture, or agricultural pressures. Based on risk quotients calculated from available sediment PNECs taken from the NORMAN ecotoxicology database, clarithromycin was also the only compound to be present at concentrations indicative of a moderate degree of environmental risk, with most of the remaining falling below this threshold. Ciprofloxacin was ostensibly found to be of a high degree of environmental risk; however, this is based on only a single sample quantified above the LoQ. Overall, antibiotic sediment concentrations suggest a low ecotoxicological risk for most of the target antibiotics, although clarithromycin, ciprofloxacin, and sulfamethoxazole warrant further monitoring in sediments.

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4.08.T-03 Evaluation of Antibiotic Mixtures through the SELECT Assay and Risk Assessment of Antibiotics in the World's Rivers

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Due to the extensive use of antimicrobials throughout the human, veterinary and agricultural sectors, antimicrobial resistance has become one of the greatest concerns to human health across the world. Though selection for antimicrobial resistance (AMR) has been proven to occur at environmental

concentrations of antimicrobials, no standardized experimental method has been designed to determine this. In the last couple of years, various predicted no effect concentrations for AMR have been suggested. However, selection for resistance some PNECs may not protect against selection and do not consider mixture effects. Therefore, this study aimed to: 1) evaluate mixtures of antibiotics using the SELECT assay and qPCR, 2) assess the potential risk of antibiotic exposure worldwide through the generation of hazard quotients, and 3) compare different sets of PNECs proposed in recent years based on various endpoints. No Observed Effect Concentrations (NOECs) were previously determined for fifteen antibiotics using the SELECT assay. A mixture of the single compound NOEC for five WWTP was then evaluated using the SELECT method. Hazard quotients were calculated using the measured concentrations of antibiotics detected by Wilkinson et al. (2022) across the world and various Predicted No Effect Concentrations (PNECs). The different PNECs used were those determined by Bengtsson-Palme and Larsson (2016) (PNECMIC), the AMRIA (PNECAMRIA) and the SELECT assay (PNECsSELECT). The mixture of NOECs for each WWTP presented a lower NOEC than the mixture of the single compound NOECs. One in seven river sites globally presented concentrations where antibiotics may be expected to be causing some effects on bacteria. Overall, the findings in this study indicate that even at concentrations where no effect is expected for antibiotics as single compounds, the combination of these may cause selective pressure on bacterial communities and may be increasing the relative abundance of certain resistance genes. Additionally, this study showed the potential hazards of antibiotics in surface waters across different continents, providing us a first glimpse into the potential impacts of AMR globally.

4.08.T-04 Flow of AMR Drivers Through the Farm Environment

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A circular economy between agriculture and organic waste streams can recycle essential resources for farming through the recovery of water, biomass, and nutrients from sanitation waste solids, effluents, and livestock manure at scale. However, one of the most challenging barriers to safely operating a sanitation-agriculture circular economy is the widespread occurrence of antibiotics and other antimicrobial agents in manure and sanitation waste and the potential role this plays in the development of antimicrobial resistance (AMR). The potential environmental and health risks of the transmission of antimicrobial molecules and ARGs in farm settings are currently unknown. To address this knowledge gap, laboratory models were used to track antibiotic resistant genes and antimicrobial residues at a bench top scale to reveal novel insights into where biological and chemical contaminants aggregate following antibiotic administration to livestock and manure reuse. Specifically the fate of a tagged blaCTX-M antibiotic resistant gene alongside amoxicillin and oxytetracycline antibiotics were tracked across a model gut soil plant continuum to help identify contaminant hotspots and thus identify where interventions need to be made. The fate of the tagged ARG and antibiotics was monitored in the porcine gut model over 4 weeks, after which the slurry was stored and then applied to soil, which was used to grow radish (*Raphanus sativus*). Quantification of ARG and chemical fate was achieved using Liquid Chromatography Mass Spectrometry and real-time PCR. Our results showed the dissemination and longevity of AMR drivers in slurry-soil-plant systems. With and without antibiotic pressure (i.e. no oxytetracycline or amoxicillin) the tagged ARG was quantified in the slurry pre-soil application and in the soil on day 0 (29.58 32.88 copies per 16S rDNA). Antibiotics were detected in the slurry (<372352 ng/g), slurry amended soil (<84.35 ng/g) and radish (<436.1 ng/g) demonstrating the persistence of antibiotics and ability to bioaccumulate in crops. The soil appears to be acting as a barrier to ARG accumulation in crops as blaCTX-M antibiotic resistant gene abundance in the radish was below quantifiable levels. Overall, our work therefore confirms that AMR constituents present in farm waste streams can accumulate in agricultural systems with the potential to crops, demonstrating pathways for human exposure via the consumption of contaminated produce.

4.08.T-05 Implementing Environmental Criteria for Antibiotics Production - Antibiotic Occurrence in Wastewater, Runoff and Impacted Water Bodies

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Antibiotic resistance is increasingly jeopardising the effectiveness of prevention and medical treatment of an increasing number of infectious diseases and is causing a high number of premature deaths worldwide.

By now, it is widely recognised that the release of antibiotics into the environment via production wastewater discharged from the pharmaceutical industry constitutes an important factor. Evidently, tackling such point sources through appropriate treatment of production wastewater would be a decisive step towards achieving a substantial reduction in antibiotic pollution and consequently in a reduction of occurrences of resistant pathogens. The here presented study addresses the overall feasibility of implementing maximum permitted API concentrations in production wastewater and how to verify compliance. Wastewater from 19 production sites from Europe, India and China has been investigated. The sites selected previously agreed to comply with the PNEC values for certain antibiotics in their wastewater and to permit independent inspections. In addition, wherever possible, supplementary environmental investigations were conducted in water bodies adjacent to the production sites.

Over 27 different antibiotics have been detected, some of them repeatedly and at several sampling locations. Antibiotic concentrations exceeding PNEC limits were found at ten production sites - both in wastewater samples and in affected environmental samples. Maximum environmental concentrations ranged from 0.1 µg/l up to 18.5 mg/l, wastewater concentrations from 0.1 µg/L to 22.5 µg/L. In the total number of environmental water samples analysed, more than 60% of antibiotic concentrations exceeded the ecotoxicological PNEC value, whereas no reliable, scientifically derived effect threshold was available for other antibiotics in these samples.

The results of our study confirm and quantify that wastewater from pharmaceutical production sites, as well as surface runoff and thus the general handling of active substances at these sites, contribute significantly to high concentrations of antibiotics in the environment and thus to the potential emergence of antibiotic resistance. Moreover, in view of the current intention to regulate the emissions of antimicrobial substances via the environmental risk assessment for human pharmaceuticals, it should be borne in mind that an effective system for verifying the values or explanations provided by the companies is required.

4.08.P Antimicrobials and Antimicrobial Resistance in the Environment

4.08.P-Tu341 Monitoring Antibiotics and Antimicrobial Resistance in an Urban Waterway

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Antimicrobial resistance (AMR), often termed the "Silent Pandemic," poses a significant global health challenge, with environmental reservoirs playing a crucial role in the evolution and transmission of resistance. Urban waterways like the Buckingham Canal (BKM) in Chennai, Tamil Nadu, India which receives 60% of untreated sewage and acts as a conduit for stormwater and various pollutants, may serve as potential hotspots for AMR proliferation.

This study investigated the presence of antibiotics, antibiotic-resistant bacteria (ARBs), and resistance genes (ARGs) in BKM, assessed their temporal and spatial variations, and explored correlations to understand AMR contributing factors. Water samples were collected at different intervals (June 2022, April 2023, June 2023, and January 2024), and sediment samples were obtained in April and June 2023 across approximately a 45-km stretch of the canal. Antibiotic concentrations were quantified via liquid chromatography-tandem mass spectrometry (LC-MS/MS) and bacterial counts by colony-forming units (CFU). Antimicrobial susceptibility testing (AST) and the Multiple Antibiotic Resistance (MAR) index measured resistance patterns in *Escherichia coli*, a fecal contamination indicator. Metagenomic sequencing identified ARGs and ARBs. Correlations among physicochemical parameters, antibiotic concentrations, and AMR indicators established a baseline understanding.

Results showed higher *E. coli* counts in water than in sediment due to sewage discharge. MAR indices exceeded 0.2 at all locations, indicating high-risk contamination. The wet season had increased CFU counts. Antibiotic concentrations exceeded 30,000 ng/L, even at less polluted sites like KVM and MBR, suggesting unexpected sources. Correlations between antibiotic concentrations and AMR indicators indicated a complex relationship influencing AMR development.

The insights gained will inform future studies on the exchange and transport of AMR elements between environmental compartments (water, sediment, air), which is essential for identifying sources, high-risk environments, and dissemination pathways to guide the development of predictive models aimed at mitigating the spread of AMR, contributing to global efforts to safeguard public health and ecosystems.

4.08.P-Tu342 Multi-Contamination and Antimicrobial Resistance Genes in a Stretch of River Danube

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Legacy and emerging pollutants are present as a mixture in river waters. Antibiotics (ABs) and antimicrobial resistance genes (ARGs) have been arising particular concern for the possible effects on ecosystems and human health, in accordance with the One-Health concept. WWTPs are recognized as the main source of river contamination for ABs and ARGs. These contaminants can cause disappearance and inhibition of some microbial species involved in key ecosystem functions promoting AB resistance spread among natural microbial populations and from them to humans, plants and animals by using river water for drinkable purposes and/or reclaimed water. The antibiotic resistance phenomenon seems linked not only to AB presence, but also to co-selection on bacteria by other pollutants (e.g. biocides, heavy metals) commonly found in surface water.

A river Danube stretch has been studying for 2 years (2023 and 2024) and was sampled during two consecutive seasons (spring and autumn) in selected site inside and outside Budapest city (1. before the city, close to the bank filtration water supplies; 2. inside Budapest city and downstream from a WWTP; 3. after the city, downstream another WWTP). Antibiotics (amoxicillin, ciprofloxacin, enrofloxacin, erythromycin, sulfamethoxazole and its main metabolite N4-smx) and several other contaminants (metals, antipyretics, anti-inflammatories, hormones, anticonvulsants, insect repellents, stimulants, disinfectants) were analysed. The resistance genes for sulphonamides (sul1 and sul2), the plasmid-mediated quinolone resistance genes (aac-(6')-Ib-cr, qnrS and qepA) and the mobile genetic element int11 were also searched for. Moreover, microbial abundance (direct counts), main pathogens (indicators of human footprint and water quality), including *Clostridium perfringens* (potential hosts of ARGs) and possible estrogenic effects (Yeast Estrogenic Screen, YES), were measured.

Overall results showed diffuse pharmaceutical residual concentrations (e.g. antibiotics, antipyretics and anti-inflammatories) in all sampling points, with seasonal differences. The most polluted point was that inside the city. The estrogenic effects also showed this point as the most polluted one.

Sulfamethoxazole and ciprofloxacin were the antibiotics found at the highest concentrations in all three sampling points. ARGs were always found, with the highest values for sulfonamides; however their presence was not directly correlated to antibiotic concentrations

4.08.P-Tu343 Monitoring Antibiotic Resistance from Hospital Effluent and Wastewater Treatment Plant to Surface Waters

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Hospitals and wastewater treatment plants (WWTP) are significant sources of antibiotic resistance, introducing resistant bacteria and genes into surrounding water systems. The environment plays a crucial role in the spread of antibiotic resistance and is a key focus of the One Health approach to tackle this global issue. To trace the route of antibiotics and antibiotic resistance genes (ARGs) from hospital sources through wastewater treatment processes and into the environment, a study was conducted in a southern region of Madrid, Spain. With approximately 350 beds, the public hospital provides healthcare services to an estimated population of 170,000. The nearby WWTP, which serves a population of over 200,000 employs secondary and tertiary treatment processes to ensure water quality prior to discharge. These processes play a critical role in mitigating the release of antibiotics and ARGs into the environment. Two sampling campaigns were carried out in early spring and summer, during which water samples were collected from hospital effluent, WWTP influent and effluent, a downstream location, and a nearby river into which the stream flows. Eight antibiotics, including macrolides, sulfonamides, fluoroquinolones, penicillins, and tetracyclines, were analyzed using liquid chromatography coupled with mass spectrometry. Six ARGs, the class I integron-integrase, and 16S rRNA gene abundance were quantified by real-time PCR.

All ARGs were detected, with gene copy numbers generally decreasing from the hospital effluent to the river.

The study will explore potential differences in antibiotic and ARG levels between early spring and summer to identify possible relationships between climatic environmental conditions and the increased

frequency of resistance in hospital effluent, WWTP influent and effluent, and receiving water bodies. Additionally, the performance of the WWTP in removing antibiotics and ARGs will be assessed to better understand the role of the WWTP in mitigating the spread of antibiotic resistance to the surrounding environment.

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4.08.P-Tu344 Dissemination of Antimicrobial Resistance Contaminants from On-Site Sewage Facilities and Potential Mitigation Strategies

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Domestic wastewater is a reservoir of contaminants related to antimicrobial resistance (AMR), including antimicrobial chemicals and AMR-related genes (e.g., antibiotic resistance genes (ARG)). On-site sewage facilities (OSSF) are used for decentralized wastewater treatment in areas without access to centralized sewage systems. In Sweden, OSSF treat ~13% of domestic wastewater. Occurrence of several organic chemicals in OSSF and receiving waters has been reported, although a substantial gap remains in understanding the role of OSSF in the proliferation and dissemination of AMR contaminants into the environment. This study aims to investigate the occurrence and dissemination of AMR contaminants in OSSF and its associated receiving groundwater, as well as the potential of biochar as a sustainable mitigation material. Wastewater and groundwater samples were collected during four different months from an OSSF, comprising a septic tank, aerated pond, and infiltration site, as well as a nearby centralized wastewater (WWTP) and drinking water treatment plants. UHPLC-MS/MS was used for chemical analysis, and high-throughput qPCR was used to quantify AMR-related genes. AMR selection risk and potential human ARG intake were also evaluated. Lab-scale experiments to assess the potential of biochar for removal of AMR contaminants were performed. Antimicrobial chemicals were less frequently found in OSSF compared to WWTP, attributed to sporadic consumption and lack of hospital or industrial discharges. Fluconazole was consistently detected in receiving groundwater but below AMR selection thresholds. Ciprofloxacin and fluconazole presented high AMR selection risk in OSSF wastewater. While septic tanks and aerated ponds removed many AMR-related genes, some (e.g., IncP_oriT, sulI) exhibited higher abundance in post-treatment. Clinically relevant ARG (e.g., blaCTX-M) were enriched in groundwater downstream of OSSF and occasionally also in a downstream private well. Biochar with high specific surface area effectively removed chemical contaminants in lab-scale experiments, while AMR-related genes were better retained with lower specific surface area, although with gene-specific variation. These findings underscore OSSF as contributors to AMR dissemination, necessitating better monitoring and regulation. Biochar may be a viable, sustainable, implementable mitigation strategy for the studied OSSF.

4.08.P-Tu345 Tracking Antimicrobial Resistance in Hyderabad's Musi River: Identifying Simple Markers for Wastewater-Driven AMR Pollution

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Antimicrobial resistance (AMR) is a silent pandemic that can be transmitted, spread and evolve in the environment. Numerous studies have been performed on waterborne AMR spread, but few have characterised the prevalence, sources, and transport of AMR in an Indian river catchment in adequate detail to inform mathematical modelling and exposure risk assessments.

Here we quantified taxonomic and AMR genes (ARGs), sensitive and AMR culturable bacteria (ARBs), and water quality conditions in water and sediment samples at ten locations along the Musi River (153 km stretch) through Hyderabad, a city renowned for antimicrobial manufacturing, to develop a hydraulic model for AMR spread the catchment.

In the dry season, absolute water column ARG and ARB abundances increased rapidly upon entering into the city, then declined downstream, whereas the increase and decline of AMR levels was more gradual in the wet season. Clustering and ordination showed that the river consisted of three stretches in terms of

AMR: upstream, city, and downstream. Most water quality and AMR indicators differed significantly between stretches and seasons ($p < 0.05$; PERMANOVA), although the relative distribution of ARGs between sediment and water column samples differed in the dry and wet seasons. Linear Discriminant Analysis showed that some water quality parameters, such as lower dissolved oxygen and elevated total nitrogen, can distinguish between AMR-polluted and less-polluted sites and might be good AMR proxies in river monitoring. Modelling showed that between 60 and 80% of water in the flowing river was untreated wastewater, suggesting wastewater releases dominate AMR in the river, not manufacturing sources.

The study proposes using DO and TN measurements as cost-effective proxies for wastewater pollution and AMR contamination in resource-limited settings, offering a practical alternative to qPCR-based monitoring. Also this work here highlights the urgent need for improved municipal wastewater management and treatment to make rivers like the Musi safer for use, such as irrigation, reducing AMR exposures across wider environments.

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4.08.P-Tu346 Antibiotic Resistance in *Lactococcus garvieae* in Italy: Temporal Dynamics and Geographic Patterns

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Piscine lactococcosis is a significant bacterial disease in aquaculture, particularly affecting rainbow trout (*Oncorhynchus mykiss*), and leads to considerable economic losses worldwide. The increasing antibiotic resistance in *Lactococcus garvieae* poses a growing challenge, reducing treatment efficacy and necessitating the monitoring of resistance patterns over time. This study analyzed 62 *L. garvieae* strains isolated in Italy between 1991 and 2021 during lactococcosis outbreaks in rainbow trout to evaluate temporal trends and explore geographical differences in resistance patterns between two river basins with documented histories of the disease.

Antibiotic susceptibility testing was conducted using the Kirby-Bauer disk diffusion method for the following antibiotics: amoxicillin, ampicillin, erythromycin, enrofloxacin, florfenicol, gentamicin, kanamycin, penicillin, spiramycin, streptomycin, oxytetracycline, tetracycline, and thiamphenicol. Inhibition zone diameters were classified as susceptible, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for aquatic animal pathogens. These categories were converted into semi-quantitative values for statistical analysis. Temporal trends (1991 1999, 2000 2010, 2011 2019, and 2020 2021) were assessed using Principal Component Analysis (PCA), while cluster PCA was applied to identify differences between strains from trout farms in the Ticino and Sile river basins. Statistical analyses were performed using R software. The results revealed notable resistance to aminoglycosides, including kanamycin, spiramycin, streptomycin, and gentamicin. Temporal PCA indicated an almost consistent resistance trend over time, though the ellipse for 2020 2021 deviated slightly, reflecting greater variability. Cluster PCA showed overlapping resistance patterns between strains from the two river basins, suggesting similar trends in antibiotic resistance. The observed resistance aligns with global reports of increasing aminoglycoside resistance among Gram-positive cocci. The observed trends highlight the need for enhanced antibiotic stewardship and the implementation of alternative strategies, such as vaccination, and improved farm biosecurity. Additionally, breeding programs focused on enhancing the genetic resistance of rainbow trout to diseases like lactococcosis could play a pivotal role in mitigating the impact of bacterial infections.

4.08.P-Tu347 Speciation/Characterisation of Chemical of Concern in Livestock Farming Systems

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Research on the impact of chemicals of concern (CoCs) like pharmaceuticals, antibiotics, pesticides, and sewage pollutants on UK freshwater quality has increased. However, livestock-derived organic matter (LDOM), which includes manure, slurry, urine, and dung, which is an equally important driver of change in freshwater quality has received insufficient attention. LDOM acts as a reservoir for CoCs from

veterinary treatments, pesticides, feed additives, and excreta management. These CoCs can persist in the environment, affecting the ecosystem and public health. There is significant knowledge gaps regarding the chemical and ecotoxicological impacts of livestock excreta, influenced by livestock type, production methods, management practices, and climate change. Livestock farming in the UK involved approximately 9.65 million cattle, 32.7 million sheep, 5.15 million pigs, and 183 million poultry in 2020. Cattle and sheep farming is predominantly located in the wetter northern and western regions, utilizing 10 million hectares of grassland, which accounts for 57% of the UK's agricultural land. It has also been reported that over 80% of oestrogen in UK rivers are from livestock production, with cattle excreting oestrogen an order of magnitude higher than humans. This study focus on 198 CoCs quantified in collected livestock excreta (farm manure, dung, slurries, and urine) from six UK cattle and sheep farms using ultra-performance liquid chromatography tandem mass spectrometry which forms part of a bigger study to understand the impact of sheep and cattle farming on UK freshwater quality under different environmental conditions..

This study identified the prevalence of antitubercular agents (isoniazid and isonicotinic acid) and macrolides (clarithromycin N-desmethyl, clarithromycin, erythromycin, and erythromycin N-desmethyl) among other antibiotic class in farm manure, dung, of sheep, dairy and beef cattle and slurries of cattle, while in the urine of the mentioned livestock, macrolides were prevalent. The detected antibiotics can cause antimicrobial resistance development, influence freshwater biota responses and pose a risk to human health through the food chain. This study reveals the range of CoCs released to the UK environment by livestock farming and provides some of the essential data required to support new, focused management and policy for livestock farming and UK freshwaters, and to inform the management of veterinary medicines in livestock husbandry.

4.08.P-Tu348 Manipulating the Antimicrobial Resistance Profile of Poultry Litter and Investigating the Effect on Microbial Community Composition and the Degradation of Trimethoprim

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The current environmental risk assessment for antimicrobials used for the treatment of poultry does not take into account their potential contribution to the development and spread of antimicrobial resistance (AMR). Research on the emergence of AMR in poultry scenarios is limited, and existing studies have not explored how resistance affects the persistence of antimicrobials in poultry litter. This experiment aims to investigate how the microbial community and resistance profile varies across different poultry farms and how the resistance profile of the microbial community in the litter influences the persistence of the commonly used antibiotic trimethoprim. In order to address the first objective of this study, the microbial community profile and bacterial and fungal diversity of three different litters from three different farms was investigated using 16S and ITS metabarcoding. The AMR profiles of each of the litters from the different farms were tested using an agar well diffusion method for antibiotic susceptibility testing. The experiment conducted to address the second objective of this study included three sample groups: one group of litters inoculated with bacteria with known resistance to trimethoprim, another with bacteria with known susceptibility to trimethoprim and a control group with no additional bacterial inoculation. All samples were dosed with trimethoprim at 10 µg/g and a degradation study was carried out over 90 days in line with the guidance published by the European Medicines Agency in 2011 and OECD320 (where appropriate). The hypothesis of this experiment was that the degradation of trimethoprim will be slower in the samples inoculated with resistant bacteria and quicker in the samples inoculated with susceptible bacteria. Initial results from this study revealed that there were statistically significant differences ($p < 0.05$) between the alpha and beta diversity of the three litters from the different farms. Initial results from the antibiotic susceptibility testing revealed differences in the zones of clearance and levels of resistance between farms. The findings from this study will improve our understanding of the role of antibiotic resistant bacteria in the persistence of antimicrobials in the environment.

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4.08.P-Tu349 Antimicrobial Resistance Profiles of *Lactococcus garvieae* and *L. petauri* Isolated from Cultured Rainbow Trout in Türkiye, Italy, Spain and Greece

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Lactococcus garvieae and *L. petauri* are etiological agents of fish lactococcosis, causing severe losses in trout aquaculture, especially at elevated water temperatures. As there are no established zone diameters or minimum inhibitory concentration (MIC) breakpoints for these bacteria, classifying *Lactococcus* spp. as resistant or susceptible to antimicrobials remains challenging. Determining epidemiological cutoff values (ECVs) is critical for assessing antimicrobial resistance and distinguishing wild-type (WT) isolates (with no detectable resistance) from non-wild-type (NWT) isolates (with resistance), which are less responsive to antimicrobial therapy.

In this study, ECVs for 14 antimicrobials were established using *L. garvieae* (n = 32) and *L. petauri* (n = 78) isolates collected from rainbow trout farms across Türkiye, Spain, Italy, and Greece during lactococcosis outbreaks from 2003-2022. The study aimed to investigate and compare (i) ECV calculated using two different approaches, (ii) genetic antibiotic resistance, and (iii) the presence of gene cassettes and capsule gene clusters. The ECOFFinder and normalized resistance interpretation (NRI) approaches were used to calculate the provisional ECVs. An evaluation of gene cassettes, capsule gene clusters, and the presence of fourteen antibiotic resistance genes (ARGs) that encode resistance against seven antimicrobial agents, was conducted.

The ECVs ranged from 1.25 µg mL⁻¹ (amoxicillin) to 80.25 µg mL⁻¹ (streptomycin). The WT and NWT isolate percentages differed between methods, with ECOFFinder generally yielding higher WT percentages. ARGs for tetracycline (tetA), β-lactamase (blaSHV, blaTEM, blaOXA), amphenicol (floR), and macrolide (emA) resistance were detected in <10% of isolates, while those for quinolones (gyrA and qnrA) and aminoglycosides (strB) were most prevalent. The presence of Class 1 and Class 2 integron cassette regions was noted in 40.9% and 33.6% of isolates, respectively, with 9.1% containing both. None of the isolates tested positive for capsule gene clusters.

Significant but weak correlations were observed between and among ARGs and MIC distributions of antibiotics. These findings facilitate the classification of WT and NWT isolates and contribute to improved surveillance of antimicrobial susceptibility patterns in aquaculture pathogens.

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4.08.P-Tu350 In-Vitro Analysis of Environmental Factors - Induced Responses in Multidrug-Resistant *Escherichia coli* Strains

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Climate changes can influence pathogenic bacteria directly by affecting their life cycles or indirectly by impacting their habitats and environments. Global temperature, precipitation, and humidity increase affect their transmission and distribution. Environmental stresses like pH, salinity, or temperature may also affect bacterial growth, replication, and physiological fitness, driving evolutionary trend-offs. One example is acquiring or losing antibiotic resistance from chemical residuals spread onto the environment. Rising antibiotic levels from anthropogenic activities and global temperature shifts highlight the need to understand how environmental factors influence bacterial survival and antimicrobial resistance. This study performed in the context of the European BlueAdapt project (<https://blueadapt.eu/>) aims to investigate the effects of environmental parameters, temperature and salinity, on antibiotic-resistance bacteria such as extended-spectrum beta-lactamases (ESBLs) *E. coli* isolated in our laboratory from the aquatic plastisphere community compared to reference *E. coli* ATCC 25922 strain. To simulate the change in bacterial growth due to the effect of climatic change, *E. coli* strains were incubated in a mesocosm model using media mimicking different salinities. To reflect the bathing water classification two initial inocula of *E. coli*, i.e., 102 CFU/100 mL and 105 CFU/ 100mL, were used and the bacterial growth was assessed daily after by CFU counting in different agar media after incubation at 25°C and 37°C. Data conducted on non-selective culture medium, i.e., Muller Hinton agar, showed that temperature did not markedly affect bacterial survival of both *E. coli* strains when grown in nutrient media. Conversely, a rapid decrease in

colony numbers was observed in media with a significantly higher salinity. Interestingly, the results obtained on selective culture medium, i.e., Tryptone Bile X-GLUC agar, showed that high temperature significantly affected the *E. coli* ATCC growth in saline buffers. In contrast, the ESBL-*E. coli* growth did not appear to be affected by synergic effect temperature-salinity showing a better adaptation to climate-induced changes. These findings highlight the intricate interplay between environmental factors in AMR survival and growth dynamics. Understanding these interactions is crucial for predicting the impacts of climatic changes on bacterial viability and antimicrobial resistance, especially in aquatic ecosystems.

4.08.P-Tu351 A Mesocosm Study to Assess the Effects of Benzalkonium Chloride on Biofilm, Phytoplankton, and Zooplankton

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Benzalkonium chloride (BAC) is a potent antimicrobial commonly used as the main ingredient in disinfectants and sanitizers, including those approved for use against COVID-19. Consequently, BAC use has increased globally in industrial, clinical, and household settings since the onset of the pandemic. This widespread usage has led to the continuous discharge of BACs into surface waters primarily via wastewater treatment plant effluents. Little is known about the effects of chronic BAC exposure on aquatic biota, particularly lower-trophic-level organisms such as biofilm, phytoplankton, and zooplankton. In the summer of 2023, an eleven-week mesocosm study was conducted at the International Institute for Sustainable Development Experimental Lakes Area (IISD-ELA) in northwestern Ontario, Canada, to assess the effects of BACs using five nominal concentrations (20, 112, 632, 3556, and 20 000 ng/L) and controls. Strips were deployed in the water column of each mesocosm to colonize with biofilm and sampled for chlorophyll a and ash-free dry mass. Integrated water samples were taken for BACs, nutrients, chlorophyll a and phytoplankton community assemblage. Zooplankton samples were collected and preserved at two- to three-week intervals and were identified to species and life stage for abundance and diversity analyses. Preliminary analyses indicate a decrease in biofilm chlorophyll a with increasing BAC concentration, but little effect on phytoplankton chlorophyll a or zooplankton abundance. Across all mesocosms, zooplankton communities were dominated by the cladoceran *Bosmina* spp., while the copepod *Tropocyclops extensus* was the most common cyclopoid. Calanoids were seldom observed. Mean dissolved oxygen was lowest in the highest treatment mesocosm (8.11 mg/L), with all others ranging from 8.69 to 8.89 mg/L. Further analyses of biofilm, phytoplankton, zooplankton and water quality endpoints are ongoing. Understanding the effects of BAC on aquatic biota, particularly lower-trophic-level organisms, is necessary to assess potential bottom-up effects this compound may have on aquatic food webs. This work is part of a larger study at IISD-ELA investigating the effects of BACs on aquatic ecosystems.

4.08.P-Tu352 Sulfamethoxazole and Copper Effects on the Plant-Microbiome System in an Agricultural Fertilized Soil

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Livestock manure and biosolids as fertilizers in agriculture can be a multi-contamination source for soil ecosystems. Several classes of antibiotics (ABs) such as sulphonamides are commonly used for preventing animal disease and their incomplete metabolism in the organism treated and subsequent excretion determine that animal manure can contain AB residues. On the other hand, copper (Cu) can also be present in soil due to its wide use as a fungicide for several crops.

In recent years, the effects of contaminant co-selection, such as co-resistance and cross-resistance phenomena, have been investigated. Particular concern is due to antibiotic and heavy metal co-presence in terms of antibiotic resistance persistence and spread in agricultural soil.

In this context, a microcosm experiment was performed with an agricultural soil from an organic farm. The soil was differently amended with manure or digestate as fertilizers and spiked with Cu and an AB (sulfamethoxazole, SMX) in presence of *Lactuca sativa*. Adding amendments, an initial increase in soil microbial activity was observed, on the other hand some ARGs increased. In the spiked conditions, the antibiotic almost completely degraded and no bioaccumulation in lettuce was found. Interestingly, SMX and Cu co-presence conditions showed an increase in the *sul2* gene, and this can be ascribed to a co-selection phenomenon. Plant-microbiome system was negatively affected by Cu in terms of vegetal biomass and microbial activity, however in digestate conditions this detrimental effect was significantly buffered. Indeed, digestate favoured microbial activity, biodiversity and lettuce growth, independently

from presence or absence of Cu. In conclusion, the active plant-microbiome system established favoured plant growth and the latter in turn positively promoted bacterial diversity and taxa indicative of a good-quality soil, confirming their synergic interactions.

4.08.P-Tu353 Antimicrobial Activity of Cadmium Sulphide Nanoparticles Against Microbial Contaminants from Wastewater Environment

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Conventional wastewater treatment technologies should treat microbial contaminants that could pose risk to human health. However, some of the treatment processes have proved ineffective. The existing problems are exacerbated by increasing antimicrobial resistance (AMR) to commonly used antimicrobial agents. There is rapid growth in research on nanomaterials, as alternative antimicrobial agents in wastewater treatment processes. The present study was aimed to treat microbial contaminants from wastewater environments using Cadmium Sulphide Nanoparticles (CdS NPs). CdS NPs were obtained from Vaal University of Technology, Natural Sciences Department, Chemistry division. Antimicrobial assay of the nanoparticles was evaluated against selected potential pathogenic bacterial species (*Escherichia coli* and *Staphylococcus aureus*) using Agar well diffusion and Minimum Inhibitory Concentration (MIC) assays. In a concentration-dependent approach (50 mg/ml, 25 mg/ml and 10 mg/ml), CdS NPs were subjected to the test organisms. In averages, the following zones of inhibition were observed against *E. coli*; 50 mg/ml (14 mm), 25 mg/ml (10 mm) and 10 mg/ml (9 mm), and *S. aureus*; 50 mg/ml (16 mm), 25 mg/ml (13 mm) and 10 mg/ml (12 mm). The following MICs were observed against *E. coli*; 50 mg/ml (3.125 mg/ml), 25 mg/ml (25 mg/ml) and 10 mg/ml (0.391 mg/ml), and *S. aureus*; 50 mg/ml (6.25 mg/ml), 25 mg/ml (6.25 mg/ml) and 10 mg/ml (no inhibition observed). Antimicrobial activity in the present study was confirmed by the formation of zones of inhibition and low MIC values. CdS NPs have shown that they do exhibit antimicrobial properties. The present study is aligned to Sustainable Development Goals 3 and 6, which champion for good health and well-being and provision of clean water for all by 2030. Nanotechnology has made substantial contribution in wastewater decontamination, and thus warrants further research.

4.08.P-Tu354 Can the Type of Inhibitor Interfere with the Adsorption Dynamics of Antibiotics in Granular Anaerobic Sludge?

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The presence of antibiotics in environmental compartments is mainly attributed to the discharge of treated effluent from wastewater treatment plants (WWTP). Biological processes are key players to achieving satisfactory removal of antibiotics in WWTP. Biotransformation and sorption to sludge are the main mechanisms identified in the removal of these compounds in biological reactors. These mechanisms occur simultaneously, but in some cases, it is necessary and advantageous to evaluate them separately. Partitioning of the biotransformation and sorption of antibiotics is commonly accomplished by inhibiting the biological activity of microorganisms to prevent biotransformation. The aim of this work is to assess whether the type of inhibitor can modify the adsorption dynamics of antibiotics in granular anaerobic sludge. Different inhibitors were evaluated to determine the concentration required for inhibition. After this, the removal of the antibiotics ofloxacin, norfloxacin, perfloxacin, ciprofloxacin, trimethoprim, sulfacetamide, sulfadimethoxine, sulfamethoxazole and sulfadiazine was evaluated using batch reactors under the following experimental conditions: (i) biomass inhibited with 8% (v/v) formaldehyde, (ii) biomass inhibited with 2% (v/v) glutaraldehyde, (iii) active biomass without substrate, (iv) active biomass with substrate, and (v) without biomass. The results indicated that the type of inhibitor can interfere with the dynamics of antibiotic removal by adsorption. This difference is more evident in antibiotics that are poorly removed by adsorption during anaerobic digestion, such as those of the sulfonamide class (sulfacetamide, sulfamethoxazole, sulfadimethoxine and sulfadiazine) and trimethoprim. For antibiotics that have a greater affinity for adsorption in anaerobic biomass, such as the fluoroquinolone class (ofloxacin, norfloxacin, perfloxacin and ciprofloxacin), the effect of the inhibitor on adsorption is lower. The initial findings suggest that the use of inhibitors to assess antibiotic adsorption should be carefully approached. First, the minimum concentration for inhibition must be determined and the feasibility of its application assessed. Secondly, the type of antibiotic must be taken into account, if it has an affinity for removal by adsorption the inhibitor will not have an effect on adsorption, otherwise the effect of the inhibitor will be greater.

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4.08.P-Tu355 Enhanced Antibiotic Removal in Anaerobic Wastewater Treatment Using Granular Activated Carbon

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Antibiotics can have a number of adverse effects on human health and the environment, requiring their removal in wastewater treatment plants (WWTPs). Anaerobic biological reactors have been highlighted as a sustainable and economically viable alternative for wastewater treatment, particularly during the methanogenic phase. This study aimed to evaluate the presence of granular activated carbon (GAC) as a conductive support material in a methanogenic hybrid reactor (R1) for the removal of antibiotics (Ciprofloxacin, Norfloxacin, Ofloxacin, Enrofloxacin, Pefloxacin, Sulfamethoxazole, Sulfadimethoxine, Sulfadiazine, Sulfacetamide, and Trimethoprim) in synthetic wastewater. To enable comparisons to be made, polyurethane foam was used as a non-conductive support material in a second reactor (R2). Both reactors had the same design characteristics and were operated in continuous flow for 182 days at four different hydraulic retention times (HRTs) (18h, 12h, 8h and 4h, orderly). Removal efficiency was evaluated using an online SPE system and liquid chromatography interfaced with mass spectrometry QTRAP 5500 HPLC-MS/MS. In general, the treatment decreased antibiotics concentration in effluents. The reduction in HRT decreased the antibiotic removal efficiency in both reactors. However, in all four operating conditions, R1 showed higher removal rates (73,5%, 65,6%, 54,6%, and 53,5% respectively) compared to R2 (47,4%, 52,2%, 41,5%, and 42,3%, respectively), revealing that the presence of conductive support material enhances antibiotic removal efficiency in anaerobic methanogenic reactors. For most antibiotics, the statistics show that there are no significant differences between the removal efficiencies of the 18h and 12h HRTs, but there are for the 12h, 8h and 4h HRTs ($p > 0.05$). These results show that the 12h HRT is the best suited to the removal of these antibiotics in methanogenic hybrid reactors containing GAC as a conductive support material, when compared to all the other operating conditions and support material. High rates of removal efficiency of chemical oxygen demand (95.86%), carbohydrate (97.05%) and protein (93.26%) were also achieved in this operating condition for R1. These findings indicate that the incorporation of conductive materials, such as GAC, can be an effective alternative for optimizing the treatment of wastewater contaminated by antibiotics, contributing to the sustainability and efficiency of treatment processes.

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4.08.P-Tu356 Advancing Antimicrobial Resistance Control: Electro-Oxidation Technology for Wastewater Treatment with Comprehensive Ecotoxicology Assessment

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The emergence and dissemination of antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs) in aquatic ecosystems represent a pressing global health concern. Conventional wastewater treatment approaches inadequately address this challenge, underscoring the urgency for innovative solutions such as electro-oxidation (EO) technology. However, the safety and sustainability of EO-treated wastewater, particularly with respect to toxicity and risk, necessitate rigorous evaluation.

This study addresses the pivotal challenge of assessing the safety and sustainability of EO technology in wastewater treatment, with a specific focus on its efficacy in eradicating ARB and ARGs while minimizing the production of toxic byproducts. Employing comprehensive toxicity and risk assessment methodologies, conducted at Goethe University Frankfurt, to evaluate EO-treated wastewater. Secondary effluent samples collected from real Sewage Treatment Plants (STPs) in Chennai subjected to electrochemical oxidation in a 50 L pilot-scale batch reactor. The reduction in bacterial counts and their Multidrug Resistance index and the reactor's efficacy in demineralizing 9 ARGs using qPCR technique were evaluated across various treatment intervals.

Results indicate EO's efficacy in eliminating ARBs, ARGs, and DNA concentrations in 45 minutes, significantly curtailing operational costs and environmental footprint by eliminating need for extra

chemicals. EO emerges as a comprehensive, eco-friendly solution, generating zero sludge. Notably, this study elucidates fluctuations in toxicity levels pre- and post-EO treatment, offering critical insights into potential toxic byproducts and their concentrations in EO-treated wastewater samples, imperative for ensuring the safe discharge of treated effluent into larger water bodies.

The study holds profound implications for policymakers and stakeholders, informing them of EO technology's efficacy in combating AMR while addressing associated risks and toxicity concerns. By shedding light on both the efficacy and toxicity aspects of EO technology, this research contributes significantly to advancing our understanding of its role in wastewater treatment and its broader implications for public and environmental health. Consequently, the findings serve as a guiding framework for devising strategies to mitigate AMR and facilitate the safe integration of EO technology into wastewater treatment facilities.

4.08.P-Tu357 Accounting for Microorganisms Yields Stricter Water Quality Criteria and Elevated Ecological Risks of Antibiotics: A Case Study of Sulfonamides in the Yangtze River Delta

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The derivation of water quality criteria (WQC) for antibiotics, such as sulfonamides, is complex and influenced by the inclusion of toxicity data from various organisms, including microorganisms. However, no definitive conclusions have been established on the extent of this influence. This study investigates how different organisms' toxicity data, including microbial data, affect the calculation of WQCs and the subsequent ecological risk assessment for sulfonamide antibiotics in the Yangtze River Delta (YRD), a region with significant antibiotic contamination. We selected 263 toxicity data points for eight common sulfonamides, including sulfamethoxazole (SMX) and sulfamethazine (SM2), to derive WQCs using the Species Sensitivity Distribution (SSD) method. Three types of WQCs were calculated: based on native species (WQC-n), a combination of native and non-native species (WQC-nn), and a combination of species and microorganisms (WQC-nnm). The inclusion of microbial toxicity data led to more conservative short-term WQCs, with values approximately 40.97% to 60.80% lower than those calculated without microbial data. Additionally, monitoring data from the past 15 years in the YRD indicated a 150% increase in notable ecological risks when using the WQC-nnm compared to WQC-n and WQC-nn, particularly under normal pollution scenarios. Despite this increase, there was a slight temporal decline in overall ecological risks observed over the monitoring period. This study underscores the importance of incorporating microbial toxicity data in the derivation of WQCs, as it leads to more protective criteria. The results also highlight the need for further research to refine WQCs, particularly in terms of more accurately reflecting real-world exposure scenarios, such as the complex mixture exposures that often occur in the environment. This study provides a critical foundation for future ecological risk assessments and water quality management practices.

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4.08.P-Tu358 Using Bayesian Networks for Antimicrobial Resistance Risk Assessment: A Conceptual Framework

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Antimicrobial resistance (AMR) poses a significant global challenge. While AMR can evolve in many ways in the environment, the water environment is often shown to be a unifying transmission pathway, with AMR often linked to discharges of inadequately treated wastewaters. Understanding and assessing the risk of AMR is complex. Factors such as limited data, epistemic uncertainties, and the lack of consensus on methodologies hinder the development of robust predictive models. Here, we explore the potential of Bayesian Networks (BNs) as a flexible and comprehensive tool for addressing these challenges.

BNs offer unique advantages for AMR risk assessment. By integrating diverse datasets, including both quantitative and qualitative information, and explicitly accounting for uncertainties, BNs provide a structured probabilistic approach that supports both predictive and diagnostic inference. Their graphical nature enables collaborative model co-construction with experts and stakeholders, fostering transparency,

credibility, and shared understanding of complex AMR dynamics.

In this work, we explore and demonstrate the utility of BN for AMR risk assessment via two interconnected strands. The first involves the application of BNs within a standard Quantitative Microbial Risk Assessment (QMRA) framework to estimate human infection risks arising from exposure to antimicrobial-sensitive and -resistant bacteria via contaminated river water; hence estimating not only the risk of illness but also if the illness is treatable or not. The proposed BBN has a modular structure with each module representing a step along the source-pathway-receptor conceptualisation. The approach is demonstrated on an Indian river catchment (Musi) characterised by high rates of antibiotic manufacturing and discharge.

The second focuses on modelling pharmaceutical pollution in a Scottish river catchment (Ugie) using detailed prescribing data, spatial wastewater treatment data, and pharma-specific properties and linking this to sub-minimum inhibitory concentration selective windows to assess the likelihood of AMR development via qualitative inference.

This work highlights the promise of BNs to navigate the multifaceted challenges of AMR risk assessment, offering a pathway to advance our understanding and inform risk management strategies. Future efforts will focus on refining this framework, incorporating empirical data, and applying it to further real-world case studies to validate its efficacy.

4.08.P-Tu359 Environmental Quality Standards for Antimicrobials: A Challenge for Regulatory Risk Assessment

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Traditionally, environmental risk assessment is concerned with protecting the health of environmental organisms, whereas threats to human health are a part of human health risk assessment. For antimicrobial substances the situation is more complex: antimicrobial substances in water can not only have adverse ecotoxicological effects on aquatic life, but they can also exert selection pressure on environmental bacteria, leading to the selection of pre-existing and even emergence of new antimicrobial resistances. The more widespread and diverse the environmental reservoir of antimicrobial resistance becomes the higher chances of infection and subsequent treatment failure for both animals and humans.

To avoid this, Predicted No Effect Concentrations (PNEC) for resistance selection have been proposed, to be applied in environmental risk assessment alongside PNEC based on ecotoxicological data. The current work compares concentrations of antimicrobial substances in Swiss waters with both ecotoxicological PNEC, as well as PNEC for resistance selection. Beyond this, these environmental concentrations will be used to reflect on the data availability of either PNEC type, how that relates to the underlying methods, and finally how the two PNEC types relate to each other and contextualize their differences.

In a broader context, the question is open as to how this particular challenge in antimicrobial risk assessment can be incorporated into regulatory risk assessment, e.g., into the derivation of Environmental Quality Standards (EQS) PNEC derived under the EU Water Framework Directive (WFD). Presently, the decision of if and how to incorporate resistance selection as an adverse outcome has been up to the individual assessors.

The present work explores one framework that has been used in EQS derivation previously, as well as suggested in guidance documents by the World Health Organization and the Antimicrobial Resistance Industry Alliance. This framework, while simple enough in its application, has several shortcomings, among which are data gaps; for some substances PNEC derivation is challenging because the necessary data is not available.

To further tackle the complexity of the regulatory risk assessment of antimicrobials, the revision of the Technical Guidance Document for EQS seems a next possible step. A collaboration bringing together experts from academia and regulatory risk assessors is needed.

4.08.P-Tu360 Do Clarithromycin, Ciprofloxacin or Tetracycline Pose a Risk for AMR Selection in the Environment? A Check via Classical PEC/PNEC Approach

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The overuse of clinically relevant antibiotics in human and veterinary medicine is contributing to the global increase in antimicrobial resistance (AMR). The environment serves as a reservoir for AMR and plays an important role in the development and spread of resistance.

Thus, there is need to develop a standardized risk assessment on the potential of antibiotics to select for

AMR in the environment. Various approaches based on existing data have been used to determine these selection concentrations. However, they have not been adequately validated by experimental data and there is no consensus on the advantages of the different methods for risk assessment.

Here we present a risk assessment approach for AMR with integration of classical exposure assessment (PECAB = predicted environmental concentration of a certain antibiotic) and a standardised effect assessment (PNECresist = predicted no effect concentration for resistance selection) resulting in a PECAB/PNECresist risk quotient.

This approach requires data on environmental threshold concentrations, i.e., antibiotic concentrations which are unlikely to select for AMR. As a basis, we conducted single-strain competition experiments with a variety of environmentally relevant bacteria and used antibiotics from 13 individual antibiotic classes. Finally, based on these data and clinical MIC (minimum inhibitory concentration) values, we developed a methodology to calculate valid minimum selection concentrations (MSCs), which also take fitness costs into account, as PNECresist values.

To illustrate our risk assessment approach, we have used the three clinically relevant antibiotics clarithromycin, ciprofloxacin and tetracycline as examples. The PECAB values were calculated as described in the European Medicines Agency guidelines on the environmental risk assessment of human and veterinary medicinal products. The PNECresist values were calculated using our newly developed online tool Cost-based MSC estimates (soon online).

The results for all three antibiotics will be presented here in form of a flow chart risk assessment approach. With regard to One Health, a standardised risk assessment of antibiotic resistance selection in the environment, with knowledge of the PNEC values at which resistance selection may occur, is crucial to identify where mitigation strategies are needed.

4.08.P-Tu361 A Risk-Based Quantitative Regulatory Approach to Implement the Assessment of AMR Spread via the Environment in Pharmaceutical Authorisation

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Antimicrobial resistance (AMR), as one of the most crucial human health problems all over the world, can only be managed if all relevant sectors (human health, animal health, environment) work together as part of a One Health approach. Therefore, the issue of AMR in the environment is now also addressed in the veterinary pharmaceutical legislation and in the draft human pharmaceutical legislation.

So far, standardised approaches for assessing the risk of AMR spread via the environment due to anthropogenic antimicrobial emissions are missing. Here, a quantitative risk-based approach on the implementation of such an assessment in the authorisation procedures for antibiotics as human and veterinary medicinal products is presented.

The approach assumes that the abundance of resistances will be increased in the environment if the antibiotic concentration in a certain habitat leads to selection.

In order to derive a predicted no effect concentration for the selection/spread of AMR via the environment (PNECresist) the research project MSC ok? was carried out to derive a method to calculate realistic environmental minimum selective concentration values (MSCs). These MSCs can directly be used as PNECresist for the assessment approach. To determine the predicted environmental concentration of antibiotic (PECAB) it is suggested to use the standardised methods described in the guidelines on the environmental risk assessment (ERA) for human and veterinary medicinal products.

In line with the ERA for human and veterinary pharmaceuticals, a quantitative risk quotient (RQ) for the risk of AMR spread via the environment due to antimicrobial emissions can be calculated by dividing the PECAB by the PNECresist. If RQ is below 1, no risk is expected, while at RQ above 1 a further selection and spread of AMR has to be assumed.

The presented approach can be used to quantitatively assess the risk for AMR being spread via the environment due to anthropogenic antimicrobial emissions. Although aspects like the risk of AMR being transferred back from the environment to humans or animals are not included in this approach the proposal can be seen as a starting point for future guidance development.

4.08.P-Tu362 The Collaborative AMR Multi-Stakeholder Partnership Platform: A Great Opportunity to Engage with Partners from Around the Globe to Address the Challenges of the

Global Risk of Antimicrobial Resistance

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Although antimicrobials play a crucial role in preventing and treating infections, the side effects are the possible antimicrobial resistance (AMR) in humans, animals and environment. AMR is a serious threat to global health, affecting not only the efficiency of treatments, increasing medical risks, but also food safety, food security and economic prosperity, as well as planetary biodiversity and ecosystems. Only a close collaboration between a diverse range of stakeholders at all levels of the One Health spectrum can be an effective tool to counter this phenomenon.

The AMR Multi-Stakeholder Partnership Platform, launched in 2022, is a quadrupartite (FAO, UNEP, WHO, WOA) collaboration on AMR and One Health. It is a global, inclusive and collaborative platform which catalyses a global movement for action against AMR by fostering cooperation between a diverse range of stakeholders at all levels across the One Health spectrum.

The inaugural Plenary Assembly of the Platform at FAO headquarters in 2023 joined more than 130 member delegates from different countries and interest groups. Subsequently, several representatives participated in high-level national and international meetings for strengthening global commitments and accelerating collective action on AMR. For the United Nations General Assembly High-Level Meeting (UNGA HLM) on AMR in New York in Sept. 2024, members of the multi-stakeholder partnership platform, led by the UNGA HLM Action Group, outlined key recommendations to address AMR, with the aim of making tangible progress. These recommendations call for concrete steps to address the growing threat of AMR using the One Health approach.

There is still a lot of work to be done, and all members of the platform are called to collaborate to achieve the next goals.

The Fourth High-Level Ministerial Conference on Antimicrobial Resistance, which took place in November 2024 in Jeddah, Saudi Arabia, was concurrent with the Second Plenary Assembly of the Partnership Platform on Antimicrobial Resistance. This occasion marked the adoption of the Jeddah Commitments, which define practical, actionable and cross-sectoral measures that stakeholders can take to address this complex health problem.

Transforming the Declaration from promise to practice will take an incredible amount of work, finance and political will. We must think outside the box and learn from each other's experiences to find impactful solutions.

4.09 Global Drinking Water Quality: Exposure to Natural and Anthropogenic Contaminants and Their Human-Health Effects

4.09.T-01 Pilot-Scale Suspect Screening and Non-Targeted Analysis with Integrated Toxicity Evaluation of Polar to Non-Polar Organic Contaminants in Tap Drinking Water from Western Oregon, USA.

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Drinking water contamination is an unbiased appraisal of the relationship between anthropogenic activity and water resources. Securing the quality and availability of drinking water is an escalating global challenge. A key hurdle in mitigating contamination is comprehensively addressing the chemical composition of tap water, as contaminants of emerging concern (CECs), population growth, and the impacts of climate change all exert influence over the predominant chemical constitution of waters. This variable composition similarly makes linking specific health outcomes to exposure difficult, though a growing body of evidence indicates that consuming contaminated drinking water may contribute to the development of chronic diseases associated with prolonged exposure to dilute mixtures of chemicals. Suspect screening (SS) and non-target analysis (NTA) utilizing high-resolution mass spectrometry (HRMS) offer robust capability for evaluating drinking water for mixtures of both known and unknown contaminants. Here we present findings from a 2022 sampling campaign where 12 tap water sources across western Oregon and 1 commercial water source were sampled and analyzed using SS and NTA paired with multi-modal extraction and chromatographic techniques prior to HRMS analysis. The amalgamation of analytical approaches targeting a range of physiochemically polar to non-polar contaminants resulted in 5,000 non-target spectral features. Tentative chemical annotations were made for over 100 features including the CEC benzothiazole-2-sulfonic acid, which was confirmed at the level 1 confidence interval. Toxicity data for the chemical annotations were obtained from the USEPA s

Cheminformatics Hazard Comparison Dashboard revealing potential developmental, reproductive, and carcinogenic risks to humans. Raw tap water samples were evaluated for toxicity using the zebrafish model, demonstrating adverse outcomes in the embryonic photomotor response (EPR) endpoint, a behavioral biomarker of neurotoxic effects of chemical exposures.

Comparison of the number of annotations made across extraction and chromatographic environments helps inform future analytical efforts in identifying the best practices for screening organic contaminants in tap drinking water. This study aims to support regulatory actions through the characterization, prioritization, and in vitro and in vivo toxicity testing of CECs and other contaminants detected in tap drinking water in Western Oregon.

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4.09.T-02 Identification and Risk Screening of Micropollutants in Processed Drinking Water

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Groundwater, a vital source of drinking water worldwide, is increasingly vulnerable to contamination from agriculture, healthcare, and industrial activities. The occurrence of various chemicals in drinking water raises health concerns, amplified by the discovery of new substances. Non-targeted screening in combination with risk evaluation offers comprehensive insights into chemical profiles in drinking water and their potential human health risks. Accordingly, a tandem-SPE analyte extraction using HLB and ENVI-Carb was performed on processed drinking water collected from the waterworks outlet. The subsequent LC-HRMS chemical analysis and data processing made the assignment of the chemical structure possible for about 278 features, which represent different chemical classes pesticides, pharmaceuticals, industrial compounds, and others. Using a qualitative risk evaluation based on the margin of exposure (MoE) and risk quotient (RQ), none of the individual compounds, whose MoE and RQ were computed, exceeded the threshold values (1000 for MoE and 1 for RQ). However, when considering the mixture of compounds detected within each sample, the computed values approached the threshold limits for both MoE and RQ. The qualitative hazard evaluation for the remaining compounds, using the USA-EPA hazard comparison chemometrics module, suggested several compounds rated with medium and high ranking levels. Therefore, the combination of NTA chemical profiling and risk evaluation helps explore not only the occurrence of unnoticed contaminants in processed drinking water but also demonstrates the human health concerns of the identified substances.

4.09.T-03 Revealing Per- and Polyfluoroalkyl Substance (PFAS) Contamination and Exposure Pathways in Suriname and Ghana

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Per- and polyfluoroalkyl substances (PFAS) are persistent pollutants with significant health and environmental implications. While global understanding of PFAS contamination is advancing, substantial knowledge gaps remain in lower- and middle-income countries such as Suriname (South America) and Ghana (West Africa), where vulnerable populations face exposure from diverse sources. In Suriname, potential sources include pesticides, firefighting foams, carpets, and plastic manufacturing, while in Ghana, e-waste processing hotspots such as Agbogbloshie and Ashaiman are likely major contributors. Despite these risks, limited data exist on PFAS levels in indoor and outdoor environments in these regions. This project addresses these gaps by evaluating PFAS exposure in Ghana and Suriname through environmental sampling of drinking water, fish, soil, and household dust from urban and rural areas. Sampling targeted high-risk and background sites to capture diverse exposure pathways. PFAS analysis of 40 compounds was performed using liquid chromatography mass spectrometry (LC-MS/MS), complemented by the Total Oxidizable Precursor (TOP) assay to assess precursor transformation. In Ghana, drinking water samples near e-waste processing areas revealed significantly higher contamination

levels compared to background regions, with legacy PFOA and other perfluorocarboxylic acids (PFCAs), including PFHxA, being prevalent. Sachet water, which is increasingly used as a drinking water source, was also included as a background reference but still showed PFAS contamination, with PFHxA being the only detected compound. Similarly, fish samples from Ghana demonstrated elevated PFAS concentrations, with tilapia showing the highest levels. PFHxA was the most abundant compound, and legacy PFOS was also detected, highlighting ongoing contamination despite its global phase-out. These findings highlight e-waste sites as significant contributors to PFAS pollution, driven by both current and legacy emissions. Ongoing analyses in Suriname focus on assessing PFAS contamination and precursor dynamics across various regions. By providing initial scoping data, this study addresses critical knowledge gaps and aims to inform evidence-based policies and mitigation strategies to reduce PFAS exposure in vulnerable populations in Suriname, Ghana, and similar regions globally.

4.09.T-04 Challenges of Managed Aquifer Recharge (MAR) - Potential Pathway of Contaminants of Emerging Concern (CECs) into Ground- and Drinking Water Systems or Effective Treatment?

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Managed aquifer recharge (MAR) is a common method to ensure ground- and drinking water quality and quantity, especially in high-populated, arid regions or areas with little aquifer storage capacity. MAR systems artificially infiltrate surface water into groundwater systems and eventually drinking water supplies, but surface waters often contain higher concentrations of contaminants of emerging concern (CECs) that could then enter groundwater systems through MAR. However, the fate of CECs in MAR systems and the controlling parameters of CEC transport within the subsurface are not well understood. We conducted a systematic literature review giving a detailed overview of (1) CEC abundance in surface waters, (2) factors controlling CEC mobility and retention in MAR systems as well as concluding with (3) the identification of key CECs that pass through the MAR system into groundwater reservoirs and drinking water supplies. During the review process, a search string in Scopus and Web of Science was used and followed the PRISMA diagram to screen 1543 papers resulting in 89 included papers. From the reviewed literature, we give recommendations to monitor and optimize MAR systems for upcoming demands of increasing water quality and quantity in a time of exponentially increasing numbers of new CECs. Those recommendations include the need for regular monitoring programs to address seasonal peaks and to achieve a good understanding of highly site-specific parameters. We further recommend the consideration of non-regulated, yet (very) mobile and (very) persistent, compounds in monitoring programs, as well as the redesign of MAR systems including pre-treatment options, treatment trains coupling several treatment steps, or strategic operational planning. This review therefore points out current challenges of CECs entering MAR systems and contributes with clear suggestions towards cost-efficient water treatment options to meet upcoming drinking water guidelines.

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4.09.T-05 A Harmonized Approach for the Conduction of Treatments and Analytics According to EFSA/ECHA GD on Water Treatment

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In the domain of plant protection product (PPP) authorization under commission regulation (EU) No. 1107/2009, one approval criterion requires to identify transformation products of active substances and their environmental metabolites that may be formed during drinking water treatment (DWT) and conduct a human health assessment.

In August 2023, EFSA and ECHA published a new guidance document on the impact of DWT processes on residues of PPP, biocides, and their environmental metabolites. For PPP, this guidance was entered into force in April 2024.

The guidance describes several individual test procedures to evaluate the transformation of active substances and their metabolites under DWT processes: 1) chlorination, 2) oxidation 3) ozonation, 4) a combined process of pre-oxidation followed by chlorination, 5) UV disinfection and 6) sand filtration. These tests are to be conducted at environmental related concentrations in the range of 1-10 µg/L, with detection and identification of possible transformation products (TPs) by non-target analysis, and concentration estimation through quantification via structurally related compounds. TPs quantified above

0.075 µg/L shall subsequently undergo structure elucidation and need to be further evaluated with toxicity studies and a dietary risk assessment.

Non-target analysis holds promise but lacks clear guidelines, particularly for data processing and filtering algorithms, hindering regulatory applicability. Additionally, suggested tests, conducted in isolated laboratory screens, deviate from real-world waterworks conditions, omitting pre- and post-treatments and potentially yielding "artificial" transformation products.

Despite their regulatory significance, the experimental tests lack essential validation and ring-testing for consistency and reliability. Even the interpretation of how to perform the individual procedures (e.g., concentration of the oxidant) is given by a quiet high flexibility. Within CropLife Europe (CLE) a harmonized approach of all individual test procedures was developed to ensure the best possible comparability and reproducibility by running these new regulatory studies at different laboratories. This presentation shares the harmonised approach and the recommendation of the CLE Group for the conduction of treatments and analytics according to the new guidance document on the impact of drinking water processes for plant protection products, biocides and their environmental metabolites.

4.09.P Global Drinking Water Quality: Exposure to Natural and Anthropogenic Contaminants and Their Human-Health Effects

4.09.P-We337 Toxic Legacy of Brazil's Mining Disasters: Micropollutants Profile and Health Risks in Drinking Water after Mariana and Brumadinho collapses

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Brazil has witnessed some of the most severe environmental disasters globally, notably the mining dam collapses in Mariana (2015) and Brumadinho (2019), both located in the state of Minas Gerais. These incidents are regarded as among the largest environmental and labor-related crimes in Brazilian history, collectively resulting in nearly 300 fatalities and causing extensive environmental and social damage. In this study we profiled the micropollutants reported in drinking water from municipalities affected by these disasters, using data from the Brazilian Water Quality Surveillance Information System for Human Consumption (SISÁGUA). Our analysis shows that Minas Gerais state accounted for approximately 70% of recorded exceedances of the maximum residual limits (MRLs) for drinking water in Brazil between 2011 and 2023, with 18,040 entries for Mariana and 5,966 for Brumadinho. Approximately 30% of these data points came from municipalities within the Rio Doce Basin, which supports a population of 3.5 million across 228 cities. Of these, 53 municipalities (serving around 1.4 million people) reported contaminants exceeding MRLs. A total of 65 micropollutants were identified, with 41 detected in at least one city. The top 10 contaminants above MRLs were: lead (3,023 occurrences), selenium (2,430), bromates (1,658), ethylbenzene (1,522), 1,2-dichlorobenzene (1,395), 1,4-dichlorobenzene (1,387), toluene (1,412), xylene (1,417), trihalomethanes (1,320), and haloacetic acids (1,173). Radioactive uranium was also detected above MRLs (157 occurrences). Most of these contaminants have guideline values set by the World Health Organization (WHO) for drinking water and represent a potential long-term risk of cancer and other health impacts. In October 2024, a judicial ruling ordered compensation for residents near the Mariana disaster site, with each affected individual to receive an equivalent of 7,000 USD, acknowledging the uncertainty surrounding long-term health impacts. Current efforts from our study focus on estimating the potential public health impacts of exposure to micropollutants above MRLs among affected populations.

4.09.P-We338 Emerging Contaminant 1,4-Dioxane: Emission Sources, Global Contamination, and Regulatory Disparities

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1,4-Dioxane, a probable human carcinogen, has raised significant environmental concerns due to its widespread occurrence in countries with both known and unknown histories of production and use. While a comprehensive study on its global emissions, environmental distribution, and regulatory frameworks is lacking. This study aims to fill this gap by examining the sources, environmental occurrence, and regulatory measures associated with 1,4-dioxane. We conducted a comprehensive analysis of existing global data on 1,4-dioxane emissions, focusing on industrial and domestic sources. Using publicly available data and emission inventories from countries like the U.S., Germany, and Japan, we quantified emissions from industrial processes, particularly surfactant manufacturing, polyester production, and ethoxylation processes. Additionally, we estimated emissions from household products based on their usage and disposal patterns, which have previously been underrepresented in studies of 1,4-dioxane. Environmental data on the presence of 1,4-dioxane in groundwater, surface water, drinking water, and

indoor air were also reviewed. Our findings indicate a shift in emission sources, from industrial activities to diffuse emissions linked to consumer products, especially in regions without a history of 1,4-dioxane production. We found that emissions from household products may exceed those from industrial sources in certain areas, highlighting the growing significance of domestic contributions to global contamination. Environmental contamination is no longer limited to groundwater, with increasing detections in surface waters and indoor air. We also reviewed regulatory frameworks across different countries, noting disparities in toxicity assessments and regulatory limits. These differences are largely due to varying interpretations of 1,4-dioxane's mode of action and limited toxicity data. This study underscores the need for more accurate emission inventories, comprehensive environmental monitoring, and clearer global regulatory standards to address the environmental and health risks posed by 1,4-dioxane.

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4.09.P-We339 Collective Challenge: Contaminant Mixtures in United States Private, Public, and Bottled Drinking Water

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In the United States, point-of-use drinking water is supplied via private tapwater (predominantly private wells), public-supply tapwater, and bottled water. Differences in management, monitoring, and messaging and lack of directly intercomparable exposure data influence the actual and perceived quality and safety of these different drinking water supplies and directly impact consumer decision making. An analysis of point-of-use drinking-water contaminant-mixture exposures and corresponding potential human-health effects of private-supply, public-supply, and bottled drinking water was conducted by aggregating exposure results and harmonizing apical health-benchmark-weighted and bioactivity-weighted effects predictions across studies conducted and published previously by this research group. Simultaneous exposures to multiple inorganic and organic contaminants of known or suspected human-health concern are common across all three drinking water supplies, with substantial variability observed in each and no systematic difference in predicted cumulative risk between the supplies. The results emphasize the need for improved understanding of the adverse human-health implications of long-term exposures to low level inorganic /organic contaminant mixtures across all three distribution pipelines.

4.09.P-We340 Per- and Polyfluoroalkyl Substances in Tapwater: A Case Study in the United States

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Drinking-water quality is a rising concern worldwide emphasizing the need to broadly assess exposures and potential human-health effects at the point-of-use. Exposures to per- and poly-fluoroalkyl substances (PFAS) are also of concern in the US and globally, however, there is limited information on PFAS in residential tapwater, especially from private-wells. We conducted a reconnaissance to compare human PFAS exposures in unregulated private-well and regulated public-supply tapwater. Tapwater from 716 locations (269 private-wells; 447 public supply) across the US was collected during 2016-2021.

Concentrations of PFAS were assessed by three laboratories and compared with land-use and potential-source metrics to explore drivers of contamination. The number of individual PFAS observed ranged from 1 to 9 with corresponding cumulative concentrations (sum of 16 detected PFAS) ranging from 0.348-346 ng/L. Seventeen PFAS were observed at least once with PFBS, PFHxS and PFOA observed most frequently in approximately 30% of the samples. Across the US, PFAS profiles and estimated median cumulative concentrations were similar among private wells and public supply tapwater. We estimate that at least one PFAS could be detected in about 45% of US drinking-water samples. These detection probabilities varied spatially with limited temporal variation in concentrations/numbers of PFAS detected. Benchmark screening approaches indicated potential human exposure risk was dominated by PFOA and PFOS, when detected. Potential source and land-use information was related to cumulative PFAS concentrations, and the number of PFAS detected; however, corresponding relations with specific PFAS were limited likely due to low detection frequencies and higher detection limits. Information generated supports the need for further assessments of cumulative health risks of PFAS as a class and in combination with other co-occurring contaminants, particularly in unmonitored private wells where information is limited or not available.

4.09.P-We341 Challenges and Gaps in the Human Health Risk Assessment of Per- and Polyfluoroalkyl Substances: Drinking Water Perspective

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The detection of per- and polyfluoroalkyl substances (PFAS) in surface water, groundwater, and drinking water at levels exceeding statutory or non-statutory regulatory limits has become a growing global concern due to the hazard posed to both environmental and public health. Some PFAS have been linked with various adverse health effects in humans including liver and developmental effects, decreased vaccine response in children, increased cholesterol levels and testicular cancer. However, there is a lack of consensus across the scientific community about the levels of PFAS that are of health concern. This paper reviews the varying approaches used by the various authoritative bodies in different countries in deriving health-based guideline values (HBGVs) for PFAS. For the continuous protection of public health, this paper also derives provisional HBGVs for a few PFAS with limited toxicological data which are monitored in drinking water under the EU and UK Drinking Water Inspectorate directives. The lack of toxicological data for the vast majority of PFAS and the inconsistencies in approaches to data interpretation across the authoritative organisations presents challenges for risk assessment. In the EU Drinking Water Directive for the sum of 20 PFAS, only 7 of the chemicals have HBGVs. Different organisations have proposed varying health-based guideline values (HBGV) and drinking water limits for the same compounds. For example, there is a > 4 order of magnitude difference in the HBGVs recommended by Health Canada and US Environmental Protection Agency for perfluorooctanoic acid or perfluorooctane sulphonic acid which demonstrates the complexities with PFAS risk assessment.

4.09.P-We342 Toxicity and Exposure Assessment of NDMA in Drinking Water: Multi-Omics Profiling using Zebrafish

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The presence of nitrosamines in drinking water has emerged as an ongoing concern, posing significant challenges to both water safety and public health. As widespread environmental contaminants with potent carcinogenic properties, nitrosamines, particularly N-nitrosodimethylamine (NDMA), remain a major focus of research due to their potential health risks. Despite this, the precise toxicity mechanisms and the full extent of their impact on human health have not been fully understood. NDMA is classified as a probable human carcinogen (Group B2) by the International Agency for Research on Cancer (IARC), and the World Health Organization (WHO) has set a guideline value of 100 ng/L for this compound in drinking water. Previous toxicological studies, often relying on in vivo models, such as Danio rerio, have highlighted the significant adverse effects of NDMA exposure. However, there is still a gap in our understanding of its toxicity at the molecular level, which is critical for assessing both environmental and human health risks.

In this study, we applied a multi-omics approach, combining transcriptomics and untargeted Nuclear Magnetic Resonance (NMR) metabolomics, to assess the metabolic and molecular impacts of NDMA exposure at different concentration levels on zebrafish. The results revealed significant alterations in both the transcriptomic and metabolomic profiles of zebrafish exposed to NDMA. NMR metabolomics identified several metabolites that were significantly affected by NDMA exposure, such as organic acids, amino acids and nucleosides. The most impacted pathways include purine and pyrimidine metabolisms and energy metabolism (enhanced glycolysis and TCA cycle activation). These alterations suggest that NDMA exposure disrupts fundamental biochemical processes, potentially leading to cellular stress, impaired metabolism, and carcinogenic effects.

This study underscores the importance of using multi-omics approaches in environmental toxicology. By capturing a broad spectrum of molecular alterations, from gene expression to metabolite levels, omics offer a deeper mechanistic understanding of how environmental contaminants like NDMA affect biological systems. The produced knowledge is essential in environmental pollutants risk assessment and for the development of more effective public health and regulatory strategies to protect human health and the environment.

4.09.P-We343 Suspect Screening and Non-Target Transformation Product Identification in Samples Derived from Drinking Water Treatment Processes

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The EFSA guidance document on the impact of water treatment processes on residues of active substances or their metabolites in water abstracted for the production of drinking water was issued in August 2023

(DOI: <https://doi.org/10.2903/j.efsa.2023.8194>). In general, the guidance covers the identification and exposure assessment of transformation products (TPs) of Biocidal and Plant Protection Products (PPPs) using experimental and in silico methods for hazard prediction. This does not just cover the effects of water treatment on the active substances but also their transformation products and their toxicological assessment.

Drinking water treatment is generally performed using chlorination, ozonation or UV treatment processes for disinfection or a combination of several processes. The guidance provides methods for simple laboratory procedures that are intended to be performed without the need for complex and expensive equipment.

Performing the laboratory tests is not the major challenge when considering this guidance, rather, it is the analysis.

The number and complexity of potential transformation products can be significant when considering the multiple processes involved. One of the most extensively used analytical methods for suspect and non-target analysis is high resolution mass spectrometry coupled with liquid chromatography.

This presentation focusses on the strategies that can be employed using liquid chromatography with High Resolution Accurate Mass Spectrometry (HRAM) to perform suspect screening and non-target identification on samples derived from water treatment processes.

4.09.P-We344 EFSA Drinking Water Treatment Guidance - Challenges of Experiments for Plant Protection Products and Biocides

Hauke Kattwinkel, Volker Wedek and Kristina Hoffmann, knoell Germany GmbH, Germany The authorisation and use of plant protection products (PPP) and biocidal products (BP) are regulated by Regulations (EU) 1107/2009 and (EU) 528/2012 to ensure a high level of protection for human health and the environment.

During the evaluation of active substances (a.s.) present in PPP and/ or BP a conclusion on the impact of water treatment processes on residues of a.s. and their metabolites in water abstracted for the production of drinking water could not be drawn so far. To fill the existing information gap, EFSA and ECHA jointly developed a new guidance document, namely Impact of water treatment processes on residues of active substances or their metabolites in drinking water.

Even not yet started, the experimental assessment of drinking water treatment processes according to the new guideline has already occurred with significant challenges. As outlined by different stakeholders, especially Contract Research Organisations (CRO), inter-laboratory reproducibility varied. Obviously, the experimental designs described in the guideline are lacking precise information and are far away from a standard study plan. For the future, there is a substantial need for harmonised and agreed approaches in testing drinking water treatment processes. This does also include the use of analytical methods to identify and quantify transformation products during water treatment processes, non-target screening vs. radio-analysis. Further, low laboratory capacities for conducting comprehensive studies and uncertain high costs associated with unclear experimental procedures have hindered progress in this area so far.

Moreover, the timelines set by the European Commission for implementing the new guidance document are tight, leaving little room for stakeholders to adapt and conduct the required experiments and potential subsequent toxicity and dietary safety risk assessments of transformation products in time. Therefore, it is highly recommended to develop a study concept management on purpose at an early stage.

This contribution should provide a critical view of the challenges associated with the experimental and analytical procedures outlined in the guidance document, including the formation and identification of transformation products during water treatment processes. By highlighting these challenges, we aim to facilitate a more informed discussion on the need for harmonised approaches in experimental assessing drinking water treatment processes

4.09.P-We345 Chemical Monitoring in Biota for the Water Framework Directive: Large-Scale Implementation in France with in situ Caging of *Gammarus* sp. (Crustacea)

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The application of WFD involves to member states of European Union two objectives: (1) assess the chemical status of water bodies, by checking their compliance with regulatory Environmental Quality Standards (EQSs) for priority substances and, (2) monitor trends of contamination. As an integrative matrix allowing measurements for many chemicals, biota-EQS have been included in WFD for bioaccumulative substances. Active biomonitoring using *Gammarus* sp. is applied in France based on a French standardized protocol. The purpose of this proposal is to give feedback on the application of this normalized method in France and illustrate how it meets the WFD requirements.

Gammarids are collected in breeding ponds, kept under laboratory conditions and calibrated before in situ exposure. After 21 days of exposure, inorganic and organic compound concentration levels were measured. These measurements are used to assess (1) compliance of water bodies towards crustaceans-EQS but also fish-EQS through their adjustment with the PULEX tool (Predict Usual Level Effects Xenobiotics) and (2) trends of contaminants based on available BBAC (i.e., bioavailable background assessment concentration).

Between 2018 and 2024, 4.231 cagings were performed (i.e., 891 stations and 102 campaigns). 98% of the caging systems were successfully retrieved. According to AFNOR-NF-T90-721, chemical analyses (at defined LOQ) were performed on 90% of stations.

In response to WFD requirements, the present study shows that in situ caging of *Gammarus* sp. has been successfully implemented in France since 2018. This method allows to (1) check compliance of water bodies to crustaceans/molluscs and fish-EQS, as illustrated for benzo(a)pyrene and PFOS compounds respectively, and (2) monitor contamination trends of historical (e.g., PCB, PHA) and more emerging (e.g., PFOS) compounds. Additionally, this method offers the opportunity to measure ecotoxicological effects in exposed gammarid as life traits and molecular markers.

4.10 Sustainable Remediation of Mining Impacts and Critical Materials Recovery

4.10.T-01 Mining Sites in Czechia as Potential Sites for Critical Element Recovery

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The Czech Republic has a long tradition of mining raw materials, and uranium has played a very prominent role. In the Middle Ages, silver was mined in many locations in Bohemia. Since 1945, uranium has been mined on an industrial scale at 23 uranium deposits. Most Czech mines were closed for ecological and economic reasons promptly after the Czech Velvet Revolution in the early 1990s. The ecological damage caused by long-term mining activity is enormous in many locations, and therefore the remediation is still incomplete and costly. Long-term human activity in the mines results in the weathering of rocks (oxidation) and releasing heavy metals into mine water. Weathering sulfide ores, common in mining sites, leads to generating acid mine drainage (AMD). With the cessation of mining, groundwater does not return to its natural state, but AMD flows (or has to be pumped out of the mine) for many decades.

The established practices in treating these acid mine waters include mainly aeration, raising the pH by lime milk, and precipitating heavy metals. The waters are treated in on-site treatment plants, which are permanent operations requiring constant supervision, the addition of chemicals, and toxic waste disposal. The whole operation is very costly and requires technologies that run for decades. Sustainable remediation of these waters, waste piles, and tailings is a challenge, especially in the possibility of simultaneous economically less demanding and sustainable remediation and recovery of critical elements in these wastes.

Within the framework of the Sustainable Remediation of Radionuclide Impacts on Land and Critical Materials Recovery (SURRI) project, a categorization of more than twenty sites under the responsibility of DIAMO s.e., was made. These are sites where uranium, precious metals, and other elements were mined. Selected sites were sampled, and the critical element content was evaluated. Further assessment was made on the possibility of recovering these elements from waste or solid samples from the sites. Experiments on the samples are underway and will be evaluated.

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4.10.T-02 A Deep Green Clean? Plant and Nature-Based Systems for Mining Wastes Risk Management and Critical Element Recovery

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Former metal mining areas present complex remediation and rehabilitation challenges, due to widespread metal contamination, and/or the presence of sulphide minerals, which may promote acid mine drainage. For uranium mining and milling tailings significant risk may also be generated by the presence of elevated radioactivity. The SURRI project is a current Horizon Europe programme aimed at developing new integrated risk management methods for legacy uranium mining and production sites, and historic metal mining sites, in Eastern Europe. The frequently large areal extent of contaminated materials at these former mining sites, coupled with the potential application (and lower application cost) of phyto- and other nature-based approaches to manage risk in both aquatic and terrestrial systems, make phytoremediation strategies potentially strong candidates for incorporation into integrated risk management and element recovery systems at these sites. This presentation outlines work within the SURRI project targeted on the application of plant or phyto-based techniques for risk management of tailings and other wastes, and critical element recovery, targeting U, Zn and As.

Initial work was performed on *Lemna* sp., due to their rapid growth potential and trace element accumulation capabilities, and their ability to be integrated into existing mining waste treatment systems such as settlement or clarification ponds, and constructed wetland areas. Locally collected *Lemna* sp. demonstrated a linear relationship between uptake and dosing concentration for U, with more complex relationships observed for As and Zn. Within-plant distribution and metal(loid) speciation was evaluated via SEM and XANES analysis. Results indicate that *Lemna* sp. are a promising candidate for recovery of critical elements from mining waters, given their widespread geographic distribution and rapid growth, particularly in final effluent polishing stages prior to final discharge. Ongoing work examines the transfer of elements between tailings and plant / rhizosphere using by Time of Flight Mass Spectrometry mapping, and the potential combination of electrokinetic remediation with phyto-extraction and critical metal recovery, with an overall aim of producing readily-implementable nature-based technologies that can be used both for effective risk management and metal recovery.

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4.10.T-03 Comparative Study Between Bacteria and Fungi Immobilized in Alginate Hydrogels for Bioremediation and Biorecovery of Selenium

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The immobilization of microorganisms in polymeric hydrogel matrices has gained attention in recent years for their application in environmental bioremediation and biorecovery of critical materials. This process, which improves cellular integrity, transport, handling and resistance against external stressors, is usually performed with bacteria and fungi due to their high metabolic diversity, rapid growth and evolution rate, and versatility.

The present study aims to compare the Se(IV) removal capacity of the bacterial species *Stenotrophomonas bentonitica* and the fungal species *Aspergillus ochraceus* (filamentous fungi) and *Rhodotorula mucilaginosa* (yeast). The obtained results show the great potential of Na-Alginate as an immobilization matrix for the tested species. Scanning electronic microscope images show that the biomass is widely distributed throughout the polymeric matrix in all cases. The Se(IV) removal experiments revealed the great capacity of *S. bentonitica* (70% Se(IV) elimination after one week of incubation) and *A. ochraceus* (97% Se(IV) removal rate after two weeks of incubation). *R. mucilaginosa* was also capable of reducing Se(IV) but the efficiency of the process is much lower to efficiently remove and transform soluble Se(IV)

into Se(0) nanoparticles with industrial interest. In all cases, amorphous Se(0) nanospheres were produced but only crystalline (monoclinical or trigonal) nanoparticles were produced by *S. bentonitica*. All these Se(IV) reduction products are widely distributed and retained inside the polymeric matrix, demonstrating the potential of these systems to be used in Se(0) nanoparticle recovery. These results highlight the great potential of bacteria and especially filamentous fungi immobilization in alginate hydrogels for removing Se(IV) from the environment and recover Se nanoparticles with industrial interest within the framework of the circular economy.

4.10.T-04 Realising the Potential - Electrokinetic Technologies for Risk Management and Critical Element Recovery at Former Mining Sites: Outputs from the HEU SURRI Project

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The SURRI project (HEU101079345) is an ongoing Horizon Europe programme aimed at developing new integrated risk management methods for legacy uranium mining and production sites (and other sites with potentially toxic trace element-containing wastes) in Eastern Europe. This presentation outlines work performed on the development of low-energy electrokinetic risk management and element recovery techniques, targeting metal-contaminated wastes from historic mining sites in the Czech Republic (here, the Zlate Hory site a polymetallic deposit exploited historically mainly for Au, with lesser Cu and Zn). Electrokinetic Remediation, EKR, involves implantation of electrodes in a substrate and the application of an electrical current to move, contain or recover contaminants through electromigration, electroosmosis, or electrophoresis. EKR is a highly flexible technique in which electrodes can be arranged in multiple ways, enabling it to work in various geometries and around site infrastructure. Importantly, EKR can work effectively in low permeability substrates which are not amenable to treatment by many existing techniques such as soil flushing or washing, e.g. low-permeability soils or tailings.

Electrokinetic systems tested and reported here used either a small diameter U-tube set-up or a larger volume cell with water reservoirs and ports for porewater measurement. Preliminary trials on the smaller U-Tube system showed rapid development of a pH gradient (due to hydrolysis of water at the electrodes), with spiked solutions showing migration of elements towards the oppositely charged electrode. In the larger test system spiking of test materials with Zn showed rapid electromigration of the Zn²⁺ ion away from the central injection point, and accumulation in the cathodic well, significantly exceeding diffusion-only migration rates. Further tests are underway with real site materials, and will be reported subsequently. These low-energy electrokinetic systems are promising tools for mining waste remediation and metal recovery, having been demonstrated previously at TRL7 and above. Ongoing work within SURRI examines up-scaling of electrokinetic treatment and recovery systems on real site materials, and integration of electrokinetic and bioremediation (including the potential biostimulation impact of electrokinetics on indigenous metal-tolerant or -recovering microbes) or phyto-recovery methods, with the aim of producing integrated technologies at TRL5 or higher.

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4.10.T-05 Sequential Treatment Chain for Removal of Iron and Selective Recovery of CRMs from Mine Effluent from Nizna Slana Ore Deposit (Eastern Slovakia)

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In February 2022 a sudden major outbreak of mine waters from the abandoned flooded siderite mine in Nižná Slaná (Eastern Slovakia) caused an ecological disaster on the Slaná River. High-concentrated mine-impacted effluents transported huge amounts of toxic heavy metals and metalloids directly to the recipient stream. The high iron content resulted in a significant red coloration of the river, which was observed several tens of km downstream and contamination crossed borders to Hungary. The permissible limits for pollution of surface water in the Slaná River have been exceeded heavily and adversely affected the living quality of the river.

The passive treatment system using anoxic limestone drains (ALD) as alkalinity generators followed by aerobic wetland was considered. Unfortunately, mine water from the deposit is highly supersaturated with respect to ferric iron (oxy)hydroxides and alkali metal jarosites. Appreciable concentrations of dissolved

Fe²⁺ or Al³⁺ would result in precipitation and coating of limestone surfaces and in rapid clogging of the interstitial spaces. In addition, high sulphate concentration of the water (> 30g/L) would cause problems with clogging of ALD drains due to gypsum precipitation.

Instead, an active water treatment approach using Fe-oxidizing bacteria and tuneable iron(III) precipitation, followed by selective recovery of metallic CRMs was proposed. This study outlines a sequential treatment chain for removal of iron in mine effluents of the Nizna Slana ore deposit, along with recover of Mn, Co and Ni.

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4.10.P Sustainable Remediation of Mining Impacts and Critical Materials Recovery

4.10.P-Mo374 Secondary Minerals Formed in Mine Impacted Waters (Smolnik-Slovakia)

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Mine impacted waters (MIW) are a serious environmental problem in many countries around the world. Specific form of MIW, so called acid mine drainage (AMD), occurs predominantly in sulphide deposits and originates from the weathering of sulphide bearing rocks. Iron- and sulphur oxidizing chemolithotrophic, acidophilic bacteria play an essential role in the weathering of sulphide minerals such as pyrite, chalcopyrite, tetrahedrite etc. They accelerate the oxidation of ferrous and sulphur entities in metal sulphides, leading to mineral dissolution and acid generation.

The precipitates themselves, iron ochre, are mainly formed on surfaces that are in contact with mine water. These are mainly coatings on stones and organic material in the stream. However, larger pieces can be observed in places where the flow slows down and the mineral (ochre) has room to form.

The main mineral in the outflow is Schwertmannite (Fe₈O₈(OH)₈ 2x(SO₄)x nH₂O). Along with elements such as iron and sulphur, also metalloids such as arsenic or antimony co-precipitate here.

Schwertmannite has an interesting reddish colour and there is a possibility of its use as a natural pigment. After drying, it is brittle and easily crushed into a fine powder. It can be easily mixed with a base medium, such as oil, and the resulting colour is intense and well applicable.

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4.10.P-Mo375 Study of Microbial Communities of Mine Drainage from the Mária Mine in Rožňava, Slovakia

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This study focuses on the microbial diversity of mine water drainage from the Rožňava mine, Slovakia, with an emphasis on non-culturable bacterial communities. We conducted a comparative analysis using two molecular approaches: 16S rRNA gene sequencing and whole-genome sequencing (WGS). Water samples were collected and subjected to high-throughput sequencing to describe the structure, diversity, and relative abundance of microbial taxa. Our results revealed the bacterial community composition based on sequencing of the V3-V4 regions of the 16S rRNA gene, highlighting key genera with the highest relative abundance, such as *Gallionella*, *Crenothrix* (Verrucomicrobia), *Bdellovibrio* (Proteobacteria), *Acidocella* (Proteobacteria), *Flavobacterium* (Bacteroidetes), and *Acidithiobacillus* (Proteobacteria). In contrast, WGS provided greater resolution of taxonomic diversity, revealing additional bacterial taxa not detected by 16S rRNA gene sequencing alone. For instance, WGS identified bacterial species such as *Methylothermobacter versatilis*, *Gallionella capsiferriformans*, and even ammonia-oxidizing archaea like *Candidatus Nitrosoarchaeum limnia*. Measured abiotic factors, including pH, metal concentrations, and temperature, can significantly impact the composition and structure of the microbial communities present. This comparative approach underscores the advantages and limitations of each method, providing valuable insights into the microbial ecology of mine-impacted environments and informing strategies for potential mine water treatment and secondary raw material recovery.

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4.10.P-Mo376 Potential for Critical Element Recovery using Autochthonous Microbial Community from Former Mining Sites

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The remediation of areas contaminated by past and ongoing mining activities remains a significant environmental challenge, necessitating innovative strategies to address the complex nature of the pollution at these sites. Traditional approaches for treating industrial waste and mine water primarily involve aeration and the precipitation of heavy metals using lime milk, which introduces calcium-rich minerals. However, these methods generate toxic sludge as a by-product. Given the limited availability of metal resources, mitigation of heavy metal pollution at former mining sites offers an opportunity to recover critical elements, aligning with the growing emphasis on circular economy principles.

Microorganisms are particularly promising for remediation of polluted areas because (1) they are ubiquitous, inhabiting diverse ecological niches worldwide, and (2) they can survive and function under extreme conditions, employing various mechanisms to protect themselves against toxic loads. This study, developed under Horizon Twinning program SURRI, aims to characterize the composition of autochthonous microbial communities in response to mine water treatment. It also explores their potential not only to tolerate the toxicity of heavy metal toxicity but also to facilitate the recovery critical metals from contaminated areas.

Initially, microbial activity was screened at several former mining sites in the Czech Republic using molecular-genetic methods. Subsequently, microbial genera were isolated from original samples using standard media. The ability of these isolates to tolerate heavy metals was evaluated through minimal inhibitory concentration assay and assessments of growth performance in the presence of toxic metals. Advanced electron microscopy techniques (SEM and STEM) were employed to verify the capacity of isolated microorganisms to process and precipitate heavy metals.

In this presentation, we will provide data on the natural microbial load at several former mining sites in the Czech Republic. The isolated bacterial representatives demonstrated varying abilities to overcome the presence of heavy metals under environmental toxic loads, both before and after mine water treatment. In summary, our findings highlight microorganisms as promising candidates for the remediation of contaminated sites and the recovery of critical elements, owing to their ubiquity, remarkable adaptability, and capacity to transform, precipitate and accumulate heavy metals.

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4.10.P-Mo377 Performance of Sediment Bacterial Communities Upon Acid Uranium Mining: Advances for In Situ Recovery Technology

Fadwa Jroundi¹, Cristina Povedano-Priego¹, Pierre Grizard², Michael Descostes³ and Mohamed Merroun¹, (1)Department of Microbiology, Faculty of Science, University of Granada, Spain, (2)ORANO Mining, France, (3)ORANO Mining; Centre de Geosciences, MINES ParisTech, PSL University, France Naturally occurring uranium (U) is a key resource in addressing the growing interest in the nuclear energy industry. Its efficient extraction is commonly achieved through acid In Situ Recovery (ISR), a process that induces alterations to the surrounding environment. Rehabilitation of ISR-impacted environment could be performed through natural attenuation, biostimulation or via bioaugmentation using indigenous microorganisms capable of acid neutralization and heavy metal immobilization. The behaviour and performance of these microorganisms under acidic conditions remain largely unknown, requiring further investigation to better understand their role in the environment restoration.

In this study, batch experiments were carried out using sediments collected from U ore at the Zoovch Ovoo deposit in Mongolia. Hipersaturation of the sediments was achieved by using synthetic aquifer water at different pHs (4.5, and 5.5). Furthermore, bacterial consortium including sulphate-reducing (SRB) and iron-reducing (IRB) bacteria, previously identified as sediment native microorganisms, were added to the different treatments. A mixture of lactate and acetate (and glycerol) was utilized as carbon sources to stimulate the growth of either sediment indigenous heterotrophic or added SRB/IRB bacteria. The microcosms were incubated under anoxic conditions at 28°C for 5 months. Black precipitates appeared

after 2 weeks of incubation only in the experiments at pH 5.5, where more reducing conditions were detected by pH and Eh monitoring. DNA extractions and 16S rRNA Illumina sequencing showed significant differences in the bacterial diversity. The predominance of *Clostridium* spp., and *Desulfovibrio* was notable at pH 5.5, while more heterogeneous distribution of genera was observed at pH 4.5, except for *Desulfotomaculum* and members of Tissierellales, especially abundant in presence of glycerol. Microscopic and spectroscopic techniques (VP-FESEM and EDX) were performed to analyse the precipitates formed in the sediments. At pH 4.5, the minerals exhibited corroded appearance, whereas at pH 5.5, numerous framboidal pyrites (FeS₂) and bacterial cells were observed covering the sediments. These outputs highlight the critical role of the indigenous bacterial community in restoring reducing conditions necessary for uranium immobilization. They also offer valuable insights into developing effective rehabilitation strategies for environments impacted by acid ISR activities.

4.10.P-Mo378 Biosynthesis of Selenium Nanoparticles by *Variovorax* sp., a Bacterium Isolated from a Former Gold and Copper Mine

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Former mining sites are a source of microorganisms with promising potential applications in biotechnology, bioremediation, and medicine. *Variovorax* sp. was isolated from sludge collected at a depth of 270 meters below ground level in the former gold and copper mine in Zlate Hory, Czechia. The strain demonstrated high metal tolerance, as evidenced by its minimum inhibitory concentrations and growth rate when exposed to selenium, copper, zinc, and lead. Subsequently, *Variovorax* sp. was further tested for its ability to synthesize elemental selenium nanoparticles (SeNPs) from selenium nitrate. The SeNPs were characterized using UV-Vis spectroscopy, transmission electron microscopy (TEM), and scanning electron microscopy (SEM). The nanoparticles exhibited irregular shapes, including round, elongated, and tooth-like forms, with sizes ranging from 100 to 200 nm and an average size of approximately 150 nm. Notably, the nanoparticles were detected inside the cells. Thus, *Variovorax* sp. shows promise for the recovery of elemental selenium from mine wastewaters.

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4.10.P-Mo379 Selenite Reduction by Bentonite Microbial Communities: Insights from a Long-Term Study for Deep Geological Repositories

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Deep Geological Repository (DGR) is the preferred strategy for the safe storage and management of high-level radioactive waste (HLW). This approach employs a multi-barrier system that includes sealing spent nuclear fuel within metal canisters, surrounded by engineered barriers such as highly compacted bentonite, and placing the whole structure within a stable geological formation. All projected scenarios for DGR suggest the probability of a long-term release of radionuclides (e.g., U-235, and Se-79) to the environment. Natural occurring microorganisms in bentonite have the potential to minimize the migration of radionuclides by promoting their immobilization. This study investigated the impact of microbial diversity on the immobilization of Se(IV) through its reduction and transformation in bentonites over a long-term experiment designed to simulate DGR conditions.

Water-saturated bentonite was treated with Se(IV) and inoculated with a bacterial consortium consisting of *Pseudomonas*, *Stenotrophomonas*, *Shewanella*, *Bacillus*, and *Amycolatopsis*. All the microcosms were incubated anaerobically for 4 years. DNA extractions and Next Generation 16S rRNA gene sequencing were conducted to analyze the shifts in microbial diversity. The results revealed that Firmicutes and Proteobacteria were the most abundant phyla. Interestingly, the presence of *Desulfobacterota* was detected, which could play a key role in Se(IV) immobilization through bioreduction. Precipitates ranging in color from orange to black were observed in Se(IV)-treated microcosms. Microscopic and spectroscopic techniques (VP-FESEM, STEM/HRTEM, EDX) were performed to characterize the Se(0) nanostructures (SeNS). Different crystalline forms of Se(0) were identified, including monoclinic (m-Se), and trigonal (t-Se) with diverse morphologies such as spheres, nanotubes, and hexagonal structures.

These results highlight the direct and indirect potential of bentonite-associated microbial communities in the immobilization of Se(IV). Through reduction to elemental selenium and subsequent biotransformation,

selenium is converted into a more stable and less biologically toxic element. These findings provide critical insights into the role of microbial activity in enhancing the long-term safety of DGRs.

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4.10.P-Mo380 A Hyphal Mat Solution? Root-like Fungal Networks for Mining Waste Remediation and Metal Bioextraction

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Mining activities in Europe have caused extensive environmental issues, including acid drainage and metal pollution, necessitating sustainable remediation strategies. Within the Horizon Europe project SURRI, this study explores fungi as a biotechnological solution for remediating metal-contaminated sites and recovering critical metals and metalloids such as Mn, Zn, Co, Cu, Pd, As, and Se. Unlike the well-studied bacterial systems, fungi offer unique advantages, including their ability to biosorb, sequester, and detoxify metals through their mycelial networks, making them promising agents for critical element recovery. Samples from uranium and gold mining sites in the Czech Republic were analyzed to isolate indigenous fungal species. These fungi were identified using ITS rRNA sequencing and characterized for their growth, tolerance, and metal accumulation capacities using advanced techniques like SEM, STEM, and ICP-MS. The fungi were tested on solid and liquid media under metal(loid) concentrations ranging from 1 to 16 mM. A total of 29 fungal strains were isolated, predominantly from the Zlaté Hory site. These fungi demonstrated high tolerance to metals, particularly Zn and Mn (4–8 mM), with moderate tolerance to metalloids such as As and Se, the latter tolerated up to 16 mM by some strains. Metal tolerance patterns (Zn = Mn > Co > Pb > Cu > As > Se) aligned with the concentration hierarchy of elements in the mining samples. Colony size decreased with higher metal concentrations, indicating a trade-off between growth and tolerance. Certain fungi exhibited selective accumulation, with Pb being sequestered efficiently at acidic pH 5 and As at neutral pH 7. Precipitation of Mn, Co, Pd, and As was observed on fungal agar plates within 7 days. These findings highlight the potential of fungi for critical element recovery due to their adaptability and selective metal sequestration. Future studies will assess their performance using real mine water and investigate fungi-bacteria combinations to enhance recovery efficiency. These advancements could lead to sustainable management of contaminated mining sites.

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4.10.P-Mo381 Circular Economy Approach for Cr⁶⁺ Adsorption from Water Using Black Carbon

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The demand for effective water treatment systems has risen significantly due to increasing industrial activity and growing environmental concerns. Heavy metal contamination, such as chromium (Cr⁶⁺), poses serious risks to both human health and ecological systems because of its high toxicity and persistence. Among the various methods available, adsorption stands out as one of the most effective techniques for removing heavy metals from water. However, adsorbents can be expensive, so the search for inexpensive yet effective alternatives has generated significant interest.

This study explores the potential of black carbon (BC), a versatile and cost-effective material commonly used in applications like plastic fillers, conductive systems, and tire reinforcements, as an efficient and affordable adsorbent for removing Cr⁶⁺ from water. Because of its distinct physical and chemical characteristics, BC has shown great promise for use in water treatment applications.

The impact of changing pH, adsorbent mass, and contact time on the removal effectiveness of Cr⁶⁺ was examined in batch adsorption experiments as part of the experimental investigation. Energy Dispersive X-ray Analysis and Field Emission Scanning Electron Microscopy were used to describe the BC material, which showed a porous structure that was conducive to adsorption. The findings demonstrated that raising BC mass considerably improves the removal effectiveness of Cr⁶⁺, with near-complete removal occurring

at 150 mg under acidic circumstances. Furthermore, the best results were obtained at acidic and neutral pH values, which achieved near-complete elimination for 150 mg in 5 minutes and over 70% clearance for lower BC masses in 10 minutes. The study shows that BC is an excellent adsorbent of Cr³⁺, particularly in acidic environments, providing a long-term option for water treatment applications. The study suggests that BC, an industrial cheap product, is an effective adsorbent for Cr³⁺, supporting circular economy principles through waste minimization and reuse. BC's potential for cost-effective, sustainable water treatment is highlighted, and future research is recommended to optimize operational conditions and further explore adsorption mechanisms.

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4.10.P-Mo382 Low-Energy Electrokinetic Remediation for Site Risk Management and Metal Recovery

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Electrokinetic Remediation, EKR, is a flexible remediation and element recovery technique that involves implantation of electrodes into a substrate and the application of an electrical current to move, contain, degrade or recover contaminants through electromigration, electroosmosis, or electrophoresis. Electrodes can be arranged in multiple ways, enabling EKR to work in various geometries and around site infrastructure. Importantly, EKR can work effectively in low permeability substrates which are not amenable to treatment by many existing techniques such as soil flushing or washing, e.g. low-permeability soils or tailings. As such, EKR has potential application for treatment of a wide variety of historic mining and metal production wastes, having been previously demonstrated at Technology Readiness Level (TRL) 7, e.g., for chromium ore processing residues. This presentation outlines and reviews previous research on EKR for wastes (and soil / sediment) treatment and element recovery at higher TRL. In particular it assesses a) the use of sacrificial steel electrodes to inject iron into substrates to generate impermeable barriers for subsurface containment and stabilisation, or reductively precipitate redox-sensitive contaminants (chromium, technetium), and b) showcases recent facility investments (via the UK's National Nuclear User Facility funding) which enable assessment of electrokinetic treatment potential for nuclear and other site wastes, and the ability of EKR to fence or contain and concentrate elements under realistic groundwater flow rates and conditions.

4.10.P-Mo383 A Sustainable Hybrid Thermo-Chemical Recycling Approach for High-Performance Graphite Regeneration from Spent Lithium-ion Batteries

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Recycling spent lithium-ion batteries (LIBs) has recently witnessed notable advancements. Current recycling techniques primarily focus on recovering valuable metals, often leaving spent graphite (SG) as secondary waste with limited attention. However, SG, characterized by its high surface area, robust structural integrity, and desirable electrochemical properties, presents an attractive resource for repurposing and new applications, addressing both environmental and economic concerns. This study employs an integrated mild acid leaching and low-temperature pyrolysis process to regenerate graphite (RG) from spent LIBs. Initially, the anode current collector was preprocessed to separate graphite from Cu foil, yielding uniform anode black powder (SG) with a particle size of <200 µm. The SG was treated with varying concentrations (10%, 30%, and 50%) of acetic acid solution to leach out lithium and other impurities. Inductively coupled plasma-mass spectroscopy (ICP-MS) analysis of the leached solutions identified the 30% acetic acid solution as the most effective, achieving >95% removal of metal impurities from SG. The regenerated graphite (RG30) treated with 30% acetic acid was subsequently subjected to thermal treatment in a muffle furnace at different temperatures (300°C, 500°C, 700°C, and 900°C) to eliminate residual impurities. Results revealed that pyrolysis at 700°C was most efficient in decomposing organic binders, such as polyvinylidene fluoride (PVDF), and electrolytes in the electrode material. Microcharacterization techniques, including X-ray diffraction (XRD) and scanning electron microscope (SEM) analyses of the RG, recovered post-thermal treatment (RG700), confirmed the successful removal of residual impurities and restoration of its microstructure. The results also indicated the carbonization of organic impurities, forming an amorphous carbon layer on the graphite surface. Subsequent TGA analysis will validate the removal of residual impurities, and the electrochemical performance of the regenerated graphite samples will be assessed to confirm their alignment with commercial graphite (CG) in terms of

initial charge capacity and retention rates. This study demonstrates an eco-friendly and scalable method for recovering high-quality graphite from spent LIBs, offering a sustainable approach to LIB recycling.

4.11 Marine and Coastal Pollution: Novel Quality Assessment Strategies and Management

4.11.T-01 Tracing Contaminants in Seafood Across the Global Ocean: Current Status and Future Management Directions

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Chemical contaminants, including pharmaceuticals, personal care products, microplastics, metals, nanoparticles, and persistent organic pollutants (POPs), pose severe risks to marine ecosystems due to their toxicity, persistence, and global prevalence. Despite efforts under the EU Marine Strategy Framework Directive (MSFD) to achieve Good Environmental Status (GES), gaps remain in addressing unregulated and emerging pollutants, compounded by inadequate legislation in many regions. This study consolidates data from 2018 to 2024 across FAO zones to assess pollutant levels, impacts on marine ecosystems and human health, and strategies for improving monitoring and regulation. A review of 42 articles on organic and 41 on inorganic pollutants, covering 20 contaminant classes such as phthalates, bisphenols, UV-filters, Cd, Pb, and Hg, analyzed data from 163 species for organic and 177 for inorganic pollutants. A total of 48,479 samples have been retrieved, providing a comprehensive view of contamination patterns worldwide. Results show widespread contamination by microplastics and organic pollutants, particularly phthalates and POPs, with hotspots in FAO Zones 61 (Western Central Pacific) and 51 (Western Indian Ocean). The Mediterranean (FAO Zone 37) showed high levels of DEHP and DiNP, worsened by its semi-enclosed geography, while the Southwest Atlantic (FAO Zone 41) exhibited elevated levels of UV-filters and pharmaceuticals. Benthic species like flathead grey mullet (*Mugil cephalus*) showed significant bioaccumulation. Inorganic contaminant levels varied by region and species. FAO Zone 27 (Northeast Atlantic) reported lower Cd, Pb, and Hg levels in predators like albacore (*Thunnus alalunga*) but elevated Pb and Hg in smaller species such as European pilchard (*Sardina pilchardus*). FAO Zone 51 (Western Indian Ocean) showed the lowest contamination overall, though localized Pb hotspots were found. FAO Zone 34 (Central-Eastern Atlantic) had high Hg levels, and the Mediterranean recorded the highest Hg concentrations, surpassing safety thresholds in species like gilt-head bream (*Sparus aurata*). These findings underscore the need for stricter regulations, enhanced waste management, and international cooperation to address pollution sources. Persistent pollutants remain critical concerns due to their resistance to degradation. Improved monitoring frameworks are essential to track emerging contaminants and ensure the health of marine ecosystems and seafood safety.

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4.11.T-02 Spatial Distribution of Per- and Polyfluoroalkyl Substances (PFAS) in Natural and Restored Intertidal Wetlands in the Scheldt Estuary

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Due to high human activity in coastal ecosystems, tidal marshes have become one of the most threatened natural ecosystems on the planet, with a significant decline in their ecosystem services as a result. The tidal marshes, flats and channel networks of estuaries each have their own distinct hydrodynamics, sedimentology and ecology which may affect the environmental fate and behaviour of pollutants. Intertidal areas may retain pollutants, hence impacting organisms that live, forage, or breed in these areas. Therefore, we investigated the spatial distribution of PFAS in sediments of intertidal areas in the Scheldt estuary (N Belgium & SW Netherlands), and to what extent this varies between different eco-geomorphic zones and between an old natural intertidal site (drowned land of Saeftinghe) and recently restored intertidal site (Hedwige-Prosperpolder; HPP), where tides were re-introduced. Furthermore, we investigated whether this distribution is affected by sediment characteristics and distance from the estuarine main channel. Associations between PFAS levels in sediments from the marshes and plants were investigated. Finally, we compared PFAS levels prior and after re-introduction of tides in the restoration site.

Our results show signs of both historical and recent PFAS emissions, and suggest that the geomorphology of intertidal areas could play a role in the spatial distribution of PFAS in sediments. In general, the

vegetation and the higher intertidal elevation in the marshes slow down currents, causing more deposition of finer sediment and higher PFAS concentrations. Distance from the main channel, grain size and organic matter content were less determining factors in the environmental fate. The re-introduction of tidal flooding in HPP leads to an enrichment with PFAS. Three PFAS were detected prior to tidal re-introduction, whereas eight PFAS were detected after. Concentrations of PFOS, PFOA and PFBS were respectively 12x, 3x and 5x higher after re-introduction. Although environmental risks in the intertidal areas were not assessed in this study, the enrichment could be beneficial for the Scheldt estuary and North Sea, as PFAS are filtered from the estuary and ecological risks in the estuary and adjacent sea are likely reduced. Moreover, removal of contaminated sediment beds and plants from intertidal areas would allow for remediation of the ecosystem, something which is more difficult when the pollution stays in the estuarine channel or sea.

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4.11.T-03 Patterns of Accumulation of Plastic Additives in Top Predator Sharks from the North Atlantic Ocean

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Millions of tons of untreated plastic enter the oceans annually, leaching harmful additives such as phthalates (PAEs), which are recognized as endocrine-disrupting chemicals. Phthalates have been detected in several marine organisms, and especially in top predators such as sharks, but their prevalence along the North Atlantic is unknown. This study aimed to evaluate the differences in PAEs accumulation in different tissues of blue sharks (*Prionace glauca*) caught in offshore waters and those captured in coastal areas. The objective was to establish a profile of the most prevalent phthalates in the marine environment by analysing their concentrations in the tissues of marine top predators. A total of 34 blue sharks were opportunistically sampled as bycatch in the North Atlantic, with muscle and liver tissues collected in situ. Eleven PAEs compounds were quantified using gas chromatography-mass spectrometry (GC-MS). Statistical analyses were conducted to assess differences in PAEs concentrations between tissue types and across geographic locations where the sharks were captured. Results showed that Di(2-ethylhexyl) phthalate (DEHP), Dibutyl phthalate (DBP), Di-n-octyl phthalate (DNOP), and Diisobutyl phthalate (DIBP) were the most abundant phthalates in both tissues, while Diisononyl phthalate (DINP) was mostly detected in the liver, likely due to differences in metabolic processing. Moreover, higher phthalate concentrations were observed in the liver compared to muscle, reflecting the liver's role in detoxification. Notably, coastal sharks exhibited greater accumulation of phthalates across both tissues, with differences between geographic regions being more pronounced for the muscle tissue, which showed a clear gradient of decreasing contamination with distance from shore. These findings highlight a link between geographic proximity to anthropogenic activities and contaminant levels in top marine predators. The study underscores the utility of muscle tissue in assessing short-term environmental contamination and of liver tissue in detecting phthalates with distinct chemical properties, and also highlights the intricate dynamics of phthalate accumulation in marine predators, providing critical insights into how environmental factors and tissue properties influence contaminant bioaccumulation, thereby enhancing our understanding of ocean pollution and its potential ecological and food web impacts.

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4.11.T-04 Minimum Necessary Copper Release Rates for Effective Antifouling Coatings in European Coastal Waters

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Vessel hulls are commonly coated with antifouling paints which release biocides directly into the surrounding water to prevent the attachment of marine organisms, thereby avoiding increased fuel consumption. Cuprous (I) oxide is the most frequently employed biocide in antifouling coatings today and acts to inhibit fouling through the release of copper ions. Antifouling products are regulated within the EU by the Biocidal Products Regulation which dictates that they should not be toxic beyond necessity, i.e., have a higher release rate of biocides than necessary for their efficacy. However, there is insufficient knowledge on the minimum required release of copper for antifouling coatings to be effective in different European waters making it difficult, if not impossible, to challenge if a product is overdosing.

In this study, the minimum necessary (or critical) release rate of copper to inhibit macrofouling was investigated in European coastal waters, with study sites in Atlantic (Arcachon Bay, France), Kattegat (Hundested, Denmark) and Skagerrak (Tjärnö, Sweden) waters. The efficacy of various antifouling coatings with differing copper contents (from 6 to 32 wt%) and their copper release rates were evaluated during a six-month field study, employing a combination of visual inspection and X-ray Fluorescence (XRF) analysis. The aim of the study was to determine the critical release rate of copper to deter macrofouling organisms at each site and to investigate whether it differs depending on the composition of the local fouling community.

The findings of this study indicate that a release rate of 7 µg/cm²/day was sufficient to inhibit macrofoulers at all three sites. Results also indicate that the critical release rate is a parameter that coating manufacturers can optimize, as the performance of the coatings was not solely dependent on the copper release rate. A release rate of 7 µg/cm²/day could serve as a benchmark for dose optimization of coatings for both the yacht and ship sectors to reduce their environmental impact.

4.11.T-05 Current Levels of OCPs Residues in Sediments: Towards an Environmental Risk Assessment for a Coastal Lagoon in the Southern Gulf of Mexico

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Coastal ecosystems in the southern Gulf of Mexico are continuously under various anthropogenic pressures, some associated with agricultural activities in the region, where organochlorine pesticides (OCPs) are still applied. This study aimed to record the current presence of these compounds in sediments and identify the influence of organic matter content and grain size on their distribution. Using standardized gas chromatography with mass spectrometry (GC/MS/MS) methods, seven of 16 pesticide residues (44%) were quantified in sediments, with endrin aldehyde being the most abundant compound; the recent entry of p,p'-DDT was also highlighted. Spearman's correlation matrix and principal component analysis (PCA) found that these pesticides' accumulation and spatial distribution in sediments were associated with fine grain size. The environmental risk assessment using the Sediment Quality Guidelines (SQGs) determined that ecotoxicological effects would occasionally be observed in the benthos. This information is complemented with additive risk quotients, suggesting that the presence of OCPs represents a low to moderate level of risk, mainly due to p,p'-DDT, and dieldrin, the latter with the most alarming individual risk quotients. The data obtained in this work could contribute to public policy actions focused on mitigating and preventing the ecosystem risk from these compounds to achieve integrated management of the anthropogenic activities carried out in this Mexican coastal lagoon.

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4.11.P Marine and Coastal Pollution: Novel Quality Assessment Strategies and Management

4.11.P-We346 Exploring the Distribution and Impacts of Chemical Pollution in Coral Reef Ecosystems

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Coral reef ecosystems are some of the most biodiverse and valuable ecosystems on the planet. Yet, they are threatened by a combination of local and global stressors, and coral reefs have been declining rapidly since the 1950s. The effects of climate change, like rising seawater temperatures and ocean acidification, as well as local impacts from eutrophication on coral reefs, are well-studied. However, the effects of the complex mixtures of water-borne substances and chemical pollutants on the health of corals (e.g., their reproduction, settlement, and growth) and reef ecosystems (e.g., structural integrity and functioning) are remarkably poorly understood. Moreover, water quality is rarely assessed before restoration efforts. This is surprising, as water quality (i.e., beyond eutrophication) is a major driver of coral reef health, alongside the detrimental impact of climate change effects. In addition to improving restoration and conservation success, coral reef water quality assessment can guide stressor management at a local scale, unlike global climate change effects. Many water quality assessment methods are available, mostly in the context of freshwater systems, but an impact assessment of complex chemical mixtures on coral reefs has not been established. Hence, there is a need for an effective and integrated water quality assessment method for the protection of coral reefs.

The combination of time-integrative sampling, advanced chemical analyses, and effect-based methods has seen rapid development for the impact assessment of complex chemical mixtures in aquatic ecosystems. We aim to tailor these methods to the particular requirements of coral reef environments to establish an effective water quality assessment strategy. Passive sampling was applied with in situ flow monitoring to obtain quantitative and representative chemical mixtures from bays and adjacent coral reefs in the Southern Caribbean. Passive sampler extracts were analyzed using chemical target- and non-target analyses to gain insight into the anthropogenic chemical diversity, identity, and distribution. Effect-based methods were applied to quantify mixture toxicity and gain insight into the toxic effects of chemicals across the coral ecosystem. This poster summarizes multiple years of research to discuss and guide the establishment of a future-proof coral reef water quality monitoring framework.

4.11.P-We347 Impacts of Chemical Pollution on Biodiversity and Biological Responses in the Archipelago Sea (Baltic Sea)

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Biodiversity is vital for the structure and function of marine ecosystems but is increasingly threatened by chemical pollution, a major driver of global biodiversity loss. This study investigated the effects of chemical pollutants on marine wildlife by combining sediment contamination analysis with biochemical biomarkers in the bivalve *Macoma balthica*, as well as biodiversity indicators including species composition and environmental DNA (eDNA) analysis.

In August 2024, a field sampling campaign was conducted targeting a well-characterized pollution gradient in the Archipelago Sea, northern Baltic Sea. Sediment samples were collected from five stations ranging from a pristine reference site in the outer archipelago to highly polluted inner stations.

Biodiversity was assessed through benthic fauna surveys and eDNA analysis of sediment samples. Stable isotope analysis of carbon and nitrogen will be performed to investigate trophic interactions and potential shifts in food web structure along the pollution gradient. Additionally, sediment samples were analyzed for chemical contaminants and physicochemical properties. Specimens of *M. balthica* were collected from each station, with their tissues analyzed for oxidative stress biomarkers, neurotoxicity, genotoxicity, cytotoxicity, and a condition index to evaluate biological effects.

Macrofaunal diversity declined along the pollution gradient, with sensitive species such as *Monoporeia affinis* likely restricted to the reference site, while tolerant species like *M. balthica* persisted across all stations. eDNA analysis is expected to show reduced genetic diversity and shifts in meiofaunal communities at polluted sites, highlighting ecosystem-level impacts. Stable isotope analysis is anticipated to reveal altered trophic structures and disrupted energy transfer in contaminated areas, while markedly increased biological effects are expected in *M. balthica* from the most polluted sites.

The integration of biomarker responses, contaminant levels, stable isotope data, and biodiversity

indicators is expected to demonstrate clear links between chemical pollution and changes in biodiversity. These findings will highlight the need for multistressor approaches to better understand and address the complex impacts of pollution on marine ecosystems.

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4.11.P-We348 Metals in Squids (*Doryteuthis sanpaulensis*) from the Cananéia-Iguape Estuarine-Lagoon System (SP), Brazil

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Human occupation in coastal areas is accompanied by several activities that are sources of pollution, including metals, potential toxic elements that can accumulate in organisms, presenting risk to animal health and, in the case of commercial species, to human health. The Cananéia-Iguape estuarine-lagunar system is a region with a history of contamination, mainly due to mining and agriculture, which is currently considered a Natural World Heritage and a RAMSAR site and its main economic activity is fishing. The objective of this study is to analyse the levels of heavy metals (As, Cd, Cr, Cu, Ni, Pb and Zn) in squids in order to compare them to the levels established in the legislation for human consumption and verify whether their consumption affects food security. A total of 64 squid samples were obtained with local fishermen in the city of Cananéia. In the laboratory, they were measured and weighted wet and the metals were extracted according to the methodology described by Trevizani et al. (2016) and analysed by Inductively Coupled Plasma Optical Emission Spectrometry and Mass Spectrometry (ICP-OES and ICP-MS). Ni was not detected. As ranged between 0,060 and 0,649 mg kg⁻¹; Cd ranged between 0,042 and 0,390 mg kg⁻¹; Cr presented values between 0,072 and 1,004 mg kg⁻¹; Cu was between 1,614 and 25,724 mg kg⁻¹; Pb ranged between 0,1 and 13,907 mg kg⁻¹ and Zn ranged between 5,024 and 27,344 mg kg⁻¹. These results were compared with the values established by the Brazilian (ANVISA, 1965, 2013) and international (FAO, 1983, 2017) legislation. It was noted all metals were at levels below those established by legislation except for chromium, three lead samples and one arsenic sample. Correlation analysis showed significant and positive relations between As and Cd with weight and length, which corroborates with studies indicating a high bioaccumulation of the latter in squids. The lack of evidence of contamination of metals in the squids studied suggests that this resource has a great food potential, as it does not pose a health risk. Furthermore, this was a pioneer study on squids in the region, allowing biomonitoring of metals and assessment of the quality of fishing resources, contributing to public health and serving as a basis for decision-making aimed at a better management and conservation of this area.

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4.11.P-We349 Occurrence, Concentration, and Risk Assessment of Phthalates in Commercial Fish Species from the Italian Coast

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Phthalate esters (PAEs) are contaminants of concern due to their widespread use and potential release into the environment. Despite their extensive use, assessment of PAEs occurrence, bioaccumulation and adverse health effects in marine organisms and humans are limited. This study aims to quantify PAEs levels in commercially important fish species across multiple trophic levels, and habitats, collected in six sampling sites along the Italian coast (Northern, Central, and Southern Adriatic Sea; Sardinian Sea; Ligurian Sea; and Tyrrhenian Sea). The aim of this study is to ascertain the concentrations of PAEs in diverse population groups potentially exposed to PAEs and to evaluate the level of pressure across the various sampling site under examination. A follow-up objective of this study is also to evaluate the potential impact of fish consumption on human health. The levels of 11 PAEs of environmental relevance (DMP, DEP, DAP, DPrP, DIBP, DBP, BBzP, DChP, DEHP, DINP and DNOP) were evaluated in the muscles of eight fish species. Regarding the risk assessment for human consumption the Hazard Quotient (HQ) and Life Time Cancer Risk (LTCR) index were evaluated. The results show that PAEs are widespread in the selected areas and species, in particular, DBP, DEHP, and DIBP are the most concentrated and present compounds representing more than 80% of the total PAEs in all species and areas, with an occurrence above the 90%. *Sardina pilchardus* exhibiting the highest values detected

among the sampling sites investigated. Otherwise, the analysis of sampling sites showed a smaller number of differences, which may indicate the widespread distribution of these contaminants across all the areas under investigation. As concern the risk assessment for human health, results showed that the consumption of the fish species analysed does not pose a risk to humans. Hazard quotients were lower than 1 in all scenarios and the LTCR was below the threshold of unacceptable risk. The known endocrine disrupting properties of the identified PAEs and the commercial value of the species analysed highlight the need for additional studies to better understand the potential impact on the organisms, the food web and humans.

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4.11.P-We350 Plasticizer Contamination in Commercially Important Fish Species from the Mediterranean Sea: Assessment of Potential Risk to Human Health

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Plastic materials contain a wide variety of additives which are not covalently bound to their structures, such as phthalic acid esters (PAEs), organophosphate esters (OPEs), and non-phthalate plasticizers (NPPs), which are easily released into the environment, affecting marine ecosystems. These additives can interact with marine organisms, including commercially important fish species such as bogue (*Boops boops*), European hake (*Merluccius merluccius*), red mullet (*Mullus barbatus*), and European pilchard (*Sardina pilchardus*). High fish consumption would increase human exposure to these compounds due to potential bioaccumulation in fish edible tissue. The limited studies focused on the occurrence of plastic additives in those Mediterranean fish species, and on the potential health risks associated with fish ingestion, highlights the need to deep into study.

The aim of this study was to assess the presence and concentration of PAEs, OPEs, and NPPs in the muscles of bogue, European hake, red mullet, and European pilchard, collected from the Ligurian Sea and Central Adriatic Sea (Mediterranean Sea). PAEs were analyzed with ultrasound assisted extraction (UAE) and dispersive solid-phase extraction (d-SPE), followed by GC-MS analysis. OPEs and NPPs were extracted using UAE and measured with a Turbulent Flow Chromatography (TFC) purification system coupled to LC-MS/MS. In addition, the potential risk to human health from fish consumption was assessed to determine whether the levels found could potentially be of concern for human health. PAEs were the predominant additives detected, with a similar distribution across both study areas. Among PAEs, bis(2-ethylhexyl) phthalate (DEHP) (156 ± 100 ng/g wet weight), dibutyl phthalate (DBP) (87.7 ± 76.3 ng/g ww), and diisobutyl phthalate (DIBP) (79.5 ± 71.3 ng/g ww) were the most abundant. Triethyl phosphate (TEP) (21.4 ± 7.88 ng/g ww) and acetyl tributyl citrate (ATBC) (1.12 ± 0.98 ng/g ww) were the OPE and NPP most abundant detected, respectively. A risk assessment based on median and 95th percentile fish consumption levels showed no significant health risks for humans (Hazard Quotient < 1), although some Life Time Cancer Risk values for DEHP for the youngest part of the population approached threshold limits.

These findings will contribute to fill the gaps regarding the presence of these additives in marine fish, highlighting further research is necessary to monitor a potential human risk via fish consumption.

4.11.P-We351 Signatures of Exposure to Persistent Organic Pollutants in Mesopelagic Fish from Northern California Current Ecoregion

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Global biodiversity is increasingly compromised by human activities, and understanding ecological

consequences of these disturbances is critical for conservation policy decisions and impact mitigation efforts. While waste such as sewage and plastic trash constantly enter the marine environment, more than 80% of the ocean is still unexplored, let alone examined for pollution impact. At 200 to 1000 meters below the surface, the ocean mesopelagic zone is characterized by episodic sinking of organic matters and migrating animals that retreat from illuminated levels during the day. This dynamic environment provides a unique opportunity for ecological assessment of potential hazardous chemical exposure. We targeted 135 organic pollutants in the livers of viperfish, owlfish, and lanternfish captured from 400 to 800 meters deep near the Oregon coast using high-performance gel permeation chromatography and gas chromatography mass spectrometry. Among the 52 compounds detected above the quantification limit, polychlorinated biphenyls (PCBs) are the most abundant type of chemicals, followed by pesticides, brominated flame retardants (BFRs), and phthalates. The most concentrated pollutant is a PCB, Cl₆ (153) at 3.70 ng/g, and the highest total concentration of PCBs in a liver is 21.85 ng/g, both detected in lanternfish. Besides PCBs, many pesticides on the Stockholm Convention's Persistent Organic Pollutants list were also detected, including aldrin, dieldrin, lindane, mirex, dichlorodiphenyltrichloroethane (DDT), and hexachlorobenzene (HCB). Although none of the 49 targeted current-use pesticides were detected, 21 of the 25 legacy organochlorine pesticides were detected, highlighting their persistence. The most abundant BFR is PBDE-17, with the highest concentration of 2.60 ng/g in lanternfish. Overall, lanternfish livers tend to contain more pollutants, likely due to their diel vertical migration that exposed them to surface-level pollution. Bis(2-ethylhexyl) phthalate (DEHP) is the only detected and one of the most used phthalates, and it was only detected in viperfish. Despite being in the largest solution on Earth and hundreds of meters from the surface, mesopelagic fish are not spared from exposures to hazardous anthropogenic pollutants. The ocean has been historically treated as a convenient sink for waste, and our data highlights that disposed persistent organic pollutants remained a looming threat to the marine ecosystem.

4.11.P-We352 Potential Risk Assessment of Microplastics Ingestion by Some Commercial Fish Species in the Northern Oman Sea, Iran

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Nowadays, microplastics (MPs) are emerging as a global contaminant, have attracted significant research attention. This study investigates the occurrence, abundance, and characteristics of microplastics in the gills, gastrointestinal tract (GIT), and skin of three commercial fish species (*Psettoodes erumei*, *Sphyræna putnamae*, and *Ephippus orbis*) from the northern Oman Sea. It aims to provide baseline data for potential ecological risk assessment, conducted for the first time in this region. A total of 784 microplastic particles were identified across all tissues examined. These MPs were characterized based on their physicochemical attributes using a stereomicroscope and Raman spectroscopy. The results showed that fibers were the most prevalent shape, and blue was the dominant color. Predominant MPs ranged in size from 200-500 µm. Polypropylene (PP) and Polyvinyl chloride (PVC) were the most common polymers detected. Risk assessment using the polymeric hazard index (PHI) was utilized to verify the hazard level of identified polymers. The overall hazard level of MP pollution was categorized I to II which poses minor to medium risks respectively. The findings of this study not only highlight the widespread nature of microplastic contamination in the study area but also provide critical baseline data. Moreover, given the potential effects of MPs on human health, fish consumers with high intake may face health risks which require further investigation.

4.11.P-We353 Comparison of Species Sensitivity Distribution Modeling Approaches for Assessing Risk of Microplastic Particles using Monitoring Data from Tokyo Bay

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In recent years, the impact of microplastics (MPs) on marine ecosystems has become a growing concern, attracting attention from governments, society, and researchers around the world. This increased awareness has led to a rise in studies on MPs in the marine environment, including monitoring their occurrence, understanding their behavior and fate, developing analytical methods, and examining their effects on ecosystems and organisms. Despite these advancements, there remains a critical gap in quantitative assessments that are essential for formulating effective, risk-based management strategies tailored to real-world scenarios. One promising approach to address this gap involves the assessment of ecological risks posed by MPs using species sensitivity distributions (SSDs). While significant progress has been made in generating toxicity data and monitoring smaller-sized MPs, the lack of standardized methodologies continues to hinder efforts, introducing substantial uncertainties in risk assessments. To address this, the present study utilizes comprehensive microplastic monitoring data from Tokyo Bay, combined with SSDs derived through multiple approaches such as a Bayesian modeling, to quantify ecological risks. Additionally, this study explores how variations in MP size classifications and differences in key assumptions influence the outcomes of these risk assessments, providing valuable insights into improving the robustness and reliability of future risk assessment frameworks with SSDs.

4.11.P-We354 ‘NurdleTrack’ – Source Identification and Hazard Assessment of Marine Plastic Nurdle Spills

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Unintentional spills of plastic nurdles from shipping containers represent the second largest source of primary microplastic to the marine environment, with an estimated annual release of >200 kTons. Given their high production volumes and natural buoyancy, polyolefins (PE and PP) are unsurprisingly the most commonly found nurdle types on beaches. With the increasing focus on nurdles as a potential environmental hazard, source identification, chemical hazard assessment, and the development of effective spill response are crucial processes.

With NurdleTrack, we offer analytical chemical tools that can simultaneously address both source identification and hazard assessment within a short response time. A multi-tiered fingerprinting approach is presented, utilizing physical characteristics followed by thermal desorption and pyrolysis GC-MS in combination with multivariate statistics to robustly assess the source of field-collected nurdles derive from any suspect source of pollution or spill event. Chemical composition determined from the same screening is further subject to in silico hazard assessment based on a combination of experimental and modelled physico-chemical and toxicological properties obtained from open-source databases.

Verification of the proposed pipeline through field and laboratory assessments will be presented. We have successfully applied the chemical fingerprinting protocol to four previous nurdle spill events (South Africa 2017 and 2020, Trans Carrier Norway 2020, Xpress Pearl Sri Lanka 2021). Validation of the hazard assessment protocol using a combination of predicted and observed toxicity of commercial and field-collected nurdles (from the Galicia spill in 2023) towards a model test species (marine microalgae *Skeletonema costatum*) and an inter-tidal test species (Common periwinkle, *Littorina littorea*) is further presented. The impact of UV-degradation on the fate and toxicity of polyolefin nurdles is assessed through accelerated degradation in the laboratory and compared to field-collected samples that have been in the environment for an extended period of time. This is critical for understanding how material changes can be integrated into source identification and to understand how response approaches can mitigate environmental impacts.

4.11.P-We355 Application of a Matrix Scoring Technique: A Reliable Methodology for the Sourcing of Macrolitter from River-Sea Interfaces?

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Marine litter and plastic pollution are a major concern and prominent topic in public discourse, science and governance. Global efforts to reduce anthropogenic litter require information about the emission sources to effectively mitigate the problem. While the scientific community seems to agree most sources are land-based and enter the ocean via sewers, stormwater overflows and rivers, there are significant

uncertainties in the quantification and identification of the sources due to the lack of established and standardised methodologies. In this work, we present how a Matrix Scoring Technique, first introduced by Tudor & Williams (2004) and adjusted by CEDRE (France), is applied as harmonised method in the TREASURE (Targeting the REduction of pLAsTic oUtfLOW into the noRth sEA) project to assess macrolitter pollution in river-sea interfaces. Our goal was to compare the sources of riverine and marine litter from the riverbanks and beaches in the area of the Yser estuary in Belgium. A source is defined here as the activity or sector that released the litter in the environment, e.g. fishing, tourism, buildings and constructions. Both the riverine and marine macrolitter batch were sorted and classified according to the Joint List of Litter Categories for Marine Macrolitter Monitoring (J-List). Each litter category was allocated to its potential source by assigning probability ratios to the entirety of all predefined source options. The developed likelihood matrix delivers an estimate of the contribution of each source activity. Each contribution was proportionally assessed, giving insights into the main sources and how they change from inland to the North Sea. A preliminary assessment indicated that the major contributions to riverine litter were from coastal tourism and recreation, hunting, and buildings and constructions whereas the beach litter was associated with coastal tourism and recreation, fish and shellfish fishing, and hunting. With the application of the Matrix Scoring Technique, we are not only applying an innovative methodology to the complex problem of litter pollution but also creating a knowledge basis for further support towards targeted governance and legislation, including the evaluation of the effectiveness of policy measures. The identification of sources is determining for the design of policies that help create safe, sustainable, and litter-free environments for current and future generations.

4.11.P-We357 Assessment of Sediment Neurotoxicity Through Effect-Based Methods: A Case Study in a Lagoon in Central Italy

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The quality of sediments in coastal lagoons is a critical environmental concern, as they can act as reservoirs for several persistent contaminants that threaten aquatic ecosystems and human health. These pollutants may originate from anthropogenic activities and natural processes and can be transferred in the foodchain. For several substances (e.g. mercury) neurotoxicity represents an important mode of action. This study investigates the neurotoxic potential of sediments from a Lagoon in Central Italy, integrating in vivo assays and biomarkers to assess the potential impact on aquatic organisms. Sediment sampling was carried out in areas of the lagoon where methylmercury was already known to be present at different concentrations in the biota. Sediment elutriates were analyzed using a behavioral assay on *Artemia salina* larvae, employing an internal laboratory method to evaluate locomotor responses, including swimming speed, distance covered, and phototactic preferences when exposed to sediment samples. Additionally, acetylcholinesterase (AChE) activity was measured in *Anguilla anguilla* specimens captured from the lagoon to assess potential neurotoxicity at the enzymatic level. Preliminary results indicate alterations in behavioural parameters of *Artemia*; these findings seem to show the presence of neurotoxic contaminants in lagoon sediments. This study highlights the importance of integrating behavioral and biochemical endpoints to evaluate the ecological risk of sediment-associated pollutants in lagoon environments emphasizing the need for continued monitoring and targeted efforts to protect these vulnerable habitats.

4.11.P-We358 Spatial Distribution of PAHs, Ni, and V, and Ecotoxicological Risk Estimation in Sediments from Terminos Lagoon Located Near a Petroleum Extraction Area in the Southern Gulf of Mexico

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The Terminos Lagoon, a Ramsar site, covers approximately 7060 km², is located adjacent to the Cantarell oil field in Campeche Sound, and is connected to the Southern Gulf of Mexico Marine Ecoregion. The objectives of this study were to determine the concentration of 16 priority polycyclic aromatic hydrocarbons (PAHs), Ni, and V in surface sediments, infer their emission sources using diagnostic ratios of PAH isomers and Ni-V, and estimate the ecotoxicological risk using the Mean Effect Range Medium Quotient (M-ERM-Q) of the three most frequent PAHs (naphthalene, phenanthrene, and benzo[a]pyrene) and Ni in sediments. PAHs ranging from 3.1 to 248.9 ng g⁻¹, Ni = 11.0-104.0 mg kg⁻¹, and V = 2.0-35.0

mg kg⁻¹ dry weight. PAHs levels were lower than in other coastal lagoons from the littoral of the southern Gulf of Mexico. PAHs were correlated to organic matter contents, and metal accumulation depended on the input of lithogenic materials. PAHs indices and metal concentrations did not indicate crude oil pollution in the area, probably due to the high hydrodynamic of the marine region of the southern Gulf of Mexico; however, pyrogenic sources of PAHs were noted. The greatest PAHs levels were detected in a site highly impacted by anthropogenic activities due that it is located in Ciudad del Carmen, the biggest urban center of the region. Ecotoxicological risk assessments pointed to an elevated risk of adverse effects in sites near swamps and mangrove zones, emphasizing the importance of continued monitoring due to the high biodiversity of these ecosystems. This research offers valuable guidance for policymakers aiming to protect the largest coastal lagoon in Mexico, the Terminos Lagoon, and other oil-affected coastal regions worldwide.

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4.11.P-We359 Polycyclic Aromatic Hydrocarbons during the 21st Century in Southern Gulf of Mexico, a Prominent Petroleum Area: A Review and Risk Estimation

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In the Southern Gulf of Mexico Marine Ecoregion (SGoME), the petroleum industry was started in 1950. Campeche Bay is notable for the presence of petroleum hydrocarbons attributed to natural seeps and oil spills. On the other hand, in several transitional waters in the SGoME, anthropogenic activities could release hydrocarbons into these environments. The objectives of this study were to present the status of polycyclic aromatic hydrocarbons (PAHs) pollution in the transitional and marine waters of the SGoME during the 21st century and estimate the ecotoxicological risk in sediments. Using a systematic search in Google Scholar, we compiled 34 published research articles and book chapters from 2000 to 2024 about the concentration of PAHs in water, sediments, and aquatic biota in the region under study. We estimate the risk quotient based on the maximum permissible concentration (RQ MPC-16PAHs) and effect range median for PAHs in sediments. The interval of PAHs concentration in water detected was from 0.001 ng L⁻¹ in La Pesca Lagoon to 370 ng L⁻¹ from Mecoaacán Lagoon. PAHs levels in sediments were higher in Sontecomapan, Mandinga, and La Mancha Lagoons in the Veracruz State, which were more significant than in other areas worldwide. The American oyster was the most studied aquatic organism for PAHs concentration in the SGoME, whose concentration interval was from 75.2 (Alvarado Lagoon System) to 5672 ng g⁻¹ (Terminos Lagoon), suggesting a difficult situation, which may imply a possible human health risk. RQ MPC-16PAHs values showed that Mecoaacán Lagoon, the Coastal shelf Tamaulipas, and Cayo Arcas Reef Complex were the three sites with the highest values from the maximum concentration detected in these sites. Veracruz State possesses the highest frequency of values higher than 1 for the RQ MPC-16PAHs. Implementing monitoring programs for PAHs is crucial to safeguard environmental and human health in petroleum regions with several sources of these pollutants.

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4.11.P-We360 Environmental Risk Assessment Associated with the Discharge of Subsea Pipeline Contents Between Oil Extraction Platforms

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This study focuses on the environmental risk assessment of the discharge of contents from a subsea pipeline connecting oil extraction platforms. The pipeline contents consist of seawater and minimal amounts of a corrosion inhibitor dosed at an initial predetermined concentration. The evaluation includes an analysis of studies on the fluid's hazard estimation, a four-year degradation test of the product, physicochemical analysis of the mentioned solution, and modeling and simulation of the discharge dilution in the receiving environment. These data are further compared with the results of ecotoxicity tests conducted on both the initial solution and the degraded solution as estimated in the decay study, before and after biodegradability tests. This approach addresses all possible scenarios of environmental exposure

for aquatic organisms, thereby reducing uncertainty in estimating the environmental risk associated with the release of the pipeline contents into the surrounding marine environment. Three risk analysis scenarios are applied, based on the concept of toxic units and species sensitivity distribution (SSD) curves, under two possible impact scenarios. Considering the worst-case discharge scenario with a specific product concentration and using the LC/EC50 value for the most sensitive species, toxic unit values are calculated as a function of dilution, factoring in pumping flow rate and ambient current velocity. Alternatively, the study considers the decline in concentration due to degradation within the pipeline during the accumulation period, providing a contrasting concentration for the SSD analysis.

4.11.P-We361 Offshore Chemical Regulatory System & Risk Assessment

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Introduction: Protection of the marine environment from the discharge of chemicals used offshore is of global importance. Sustainable operations in the oil and gas sector requires a regulatory framework ensuring protection of the ecosystem and prevention of deleterious environmental impact.

Chemicals use and discharge for petroleum exploration activities in the OSPAR region require chemicals to be registered via the OSPAR's Harmonised Mandatory Control System (HMCS). The objective of the HMCS is to protect the marine environment by identifying those chemicals used in offshore oil and gas operations with the potential for causing an adverse environmental impact and restricting their use and discharge to the sea. Thus, for the chemical supply industry there is pressure to develop products that comply and fulfil technical performance and environmental risk criteria, Operators (oil companies) requirements are more linked to ensure optimum operational performance.

On the environmental regulatory front Recommendation 2012/5 requires Contracting Parties to implement a risk-based approach (RBA) for the management of produced water discharges from offshore installations. RBA utilises a stepwise screening tiered approach involving either whole effluent toxicity and/or substance-based approach with use of dispersion modelling to establish if chemical discharges are likely to result in adverse effects to the marine environment.

Does this approach regarding methodology and regulatory framework criteria address the evaluation of potential combined effects of chemicals? Does the regulatory implementation require field monitoring to assess the impact on the ecosystem?

4.11.P-We362 The Effects of Water Accommodated Fraction from Chemically Dispersed Marine Gas Oil on the Lipid and Astaxanthin Profiles of the North Atlantic Copepod *Calanus finmarchicus*

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Copepods are key members of the zooplankton communities. Through their diets, some copepods species accumulate the carotenoid astaxanthin and lipids, which both are beneficial for copepods. For example, astaxanthin has antioxidant and anti ultra-violet radiation properties. Astaxanthin can also be extracted to be commercially used as an antioxidant supplement for human consumption. Additionally, lipid accumulation is crucial for the copepod's wellbeing and for surviving diapause during winter. However, little research is available on how copepods can utilize the astaxanthin properties to protect against pollution stress or how pollution can affect astaxanthin and lipids incorporation in copepods. Therefore, the objective of this study was to investigate the how environmentally relevant pollution, can influence lipid and astaxanthin accumulation and metabolism in the cold water copepod *Calanus finmarchicus*. The selected pollutant used in this study was a Marine Gas Oil Water Accommodated Fraction mixed with a commercial dispersant: Finasol OSR 52 (MGO CEWAF). Adult copepods were exposed for 96 hours to 4.22% MGO CEWAF. Pools of the exposed copepods were taken at 0, 24, 48, 72 and 96 hours post-exposure. The profiles of the astaxanthin and lipids were measured in pools of 10 copepods using high-resolution Mass spec orbitrap (HR-MS orbitrap). The astaxanthin profile in both exposed and non-exposed copepods was mostly dominated by monoesters (>95%), followed by free form Astaxanthin (2-4%), and diester forms (>1%). With respect to the two most abundant monoesters (C16:0 & C18:1), both showed the same dynamics throughout the experiment: Slight increase in the experimental groups (exposed and non-exposed) after 24 hours. In the the control group, compared to the concentrations at 24 hours, there was a significant decrease in the concentration of the astaxanthin after 96 hours. This decrease overtime in the non-exposed group could be due to the breakdown of the monoester astaxanthin due to starvation. On the other hand, in the exposed group, no significant changes in the concentration of the monoesters occurred throughout the experiment. This indicate that the exposure to MGO CEWAF disrupted the breakdown dynamics of the monoesters as a function of exposure time. However, the upcoming lipid profile results will further demonstrate the dynamics between astaxanthin accumulation during exposure and lipid metabolism and accumulation in *Calanus finmarchicus*.

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4.11.P-We363 Fourth Generation Oil Spill Dispersants Applied on Patagonian Petroleum Spills
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The final formulation proposed as fourth-generation dispersants was developed. We considered the applications of dispersant formulations, aiming to achieve an optimal combination of compounds based on three key characteristics, which also served as control parameters: efficacy, biodegradability, and ecotoxicity.

The new dispersants were tested on the following types of crude oil extracted from Patagonian wells in Argentina: Caleta Córdoba, Caleta Olivia, Hydra Total, and Cruz del Sur YPF. Their performance was compared with that of other commercial dispersants.

Dispersants are specially designed mixtures for oil spill response, composed of detergent-like surfactants dissolved in various organic solvents. While dispersants do not remove oil from water, they work by breaking the oil into small droplets, facilitating natural degradation processes.

After application, the aquatic phase was evaluated for ecotoxicity using two aquatic species: *Cnesterodon decemmaculatus* and *Artemia franciscana*. Sedimented oil droplets were assessed using bioassays with *Hyalella curvispina*. Relationships between the control parameters and the composition of the mixed formulations were evaluated for all the tested dispersants.

4.11.P-We364 Chemical Mapping and Hazard Assessment of Antifouling Coating Systems on the EU Market

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When ships and boats are immersed in water, the attachment of fouling organisms on ship and boat hulls can happen and consequently increasing the frictional drag of vessels and fuel consumption. The most common way to prevent fouling on vessel hulls is the application of antifouling coatings, in which copper-based biocidal coatings account for the majority of the market share. However, the usage and release of copper to the marine environment has shown to be substantial, particularly in semi-enclosed environments. Several alternative systems to copper coatings are currently available on the market, which can generally be divided into four different categories: 1) non-copper biocide coatings, 2) foul-release coatings, 3) biocide-containing foul-releasing coatings and 4) hard epoxy coatings. Despite their lack of copper, these alternative coating systems may result in other kinds of emissions into the marine environment, e.g. biocides, persistent/toxic substances and microplastics (MPs). Notably, the current reporting and evaluation processes only focus on the emission of biocides when considering the introduction of a product to the European market. However, little or no information exists regarding emissions of substances such as MPs or persistent/toxic compounds, which do not fall under the legal classification of biocides. Without this knowledge, it is difficult to decide whether the alternative coating systems have a lower environmental impact, and their efficacy also needs to be further evaluated. Hence, this calls for an answer to the question: which copper-free alternative is the most sustainable choice in comparison to traditional copper coatings for ship trafficking in European waters? So far, a comprehensive evaluation of any hull coating in this regard remains absent. To address this issue, this study investigates chemicals in antifouling coating formulations that could be released into the marine environment and compares the hazards of different coating systems.

4.11.P-We365 Risk Assessment of Metals Released from In-Water Hull Cleaning in Coastal Environment: Evaluating Metal Leaching from Particulate Wastes and the Impact of Simultaneous Operations in Harbor

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In-water cleaning (IWC) is a widely employed method for removing biofouling from ship hulls, aiming to prevent the spread of invasive aquatic species and improve vessel efficiency by reducing hull friction and fuel consumption. With the growing application of IWC, it is critical to evaluate its environmental implications, particularly concerning the discharge of cleaning effluents that may contain hazardous substances from antifouling paints. This study investigates the chemical contamination risks associated with multiple IWCs conducted simultaneously within a harbor, focusing on both the direct release of untreated effluents and the leaching of metals from paint particles into the environment. Environmental

concentrations were predicted using the Marine Antifoulant Model to Predict Environmental Concentration (MAMPEC), applying worst-case release rates of 8.07 and 11.7 $\mu\text{g}/\text{cm}^2$ for dissolved copper (Cu) and zinc (Zn), respectively, based on metal concentrations measured in effluents from local IWC trials. The model also incorporated the contribution of particulate metal emissions by examining the leaching behavior of Cu and Zn from paint particles in the water column, with estimated values of 268 μg Cu and 2213 μg Zn per gram of particles. To determine the maximum demand for IWC in the port, historical vessel statistics provided by the local port authority were analyzed, categorizing vessels by size and calculating the average daily number of vessels berthing over the past decade. Risk characterization ratio (RCR) for Cu was 1.63, indicating a potential risk to marine ecosystem, while the RCR for Zn was less than one, suggesting a low risk from zinc emissions. The risk assessment highlights the need for effective recovery or treatment of effluents to mitigate environmental risk. Regulating and managing the scale of cleaning activities based on comprehensive, site-specific risk assessments is essential to protect local marine ecosystems.

4.11.P-We366 Advances in the Detection of Organic Substances in Seawater Using an Improved Vortex-Assisted Liquid-liquid Microextraction (VLLME) Approach

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Marine ecosystems such as tropical coral reefs are threatened among others by organic substances introduced by agricultural runoff, wastewater discharge and/or recreational activities. These compounds include pesticides, pharmaceuticals, and personal care products (e.g., UV filters), with water solubilities ranging from mg L^{-1} to ng L^{-1} and octanol-water partitioning coefficients (Log Pow) from ~ 1 to > 5 . Highly lipophilic substances may raise in combination with persistency an environmental concern, even if they are present in the environment at low concentrations. The accurate detection of such substances in both standardised laboratory experiments with corals and even environmental samples through analytical monitoring at such low concentrations (e.g., down to the pg L^{-1} to ng L^{-1} range) is needed.

Traditional methods like solid-phase extraction (SPE) can be cost-intensive and inefficient. Vortex-assisted liquid-liquid microextraction (VLLME) offers a simpler and proficient alternative with high extraction efficiency for moderate to poorly water-soluble compounds. By using a dispersing solvent, VLLME further enhances extraction across an even broader range of polarities/solubilities, making it a sensitive, efficient and fast additional approach for the analysis of organic compounds in seawater (hereafter: VDLLME).

With this study, we are extending the application of VLLME and VDLLME methods to seawater samples containing UV filters (e.g., benzophenone-3, BP-3; octyl-methoxycinnamate, OMC) and pesticides (e.g., diuron, DCMU), as the VDLLME method was mainly adapted for freshwater studies, with only limited applications for marine samples. Initial results indicate that the method presented is suitable to detecting both low substances concentrations in standardised (chronic) aquatic toxicity tests with corals and to analyse environmental concentrations.

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4.11.P-We367 Automated Identification of Histological Lesions in Fish Liver for Environmental Assessment

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Stressors and pollutants often trigger both short- and long-term responses in the liver, the main site of contaminant detoxification in fish. Histopathological analysis of liver tissue is a long-standing method used to assess exposure levels in fish populations and serves as a proxy for organism health. However, dwindling numbers of trained histopathologists are unable to process ever-increasing amounts of data, at a time where such work is crucial in understanding environmental effects of climate change and rising pollution. New machine-learning (ML) tools for image identification and classification could help analyse samples more efficiently and objectively.

In this study, we selected and trained ML models to identify and quantify four common, environmentally relevant lesions in fish liver and compared results with analyses from trained pathologists. Whole-slide images were obtained from two fish species commonly used as bioindicators, *Gadus morhua* and *Limanda limanda*. For each image, a small area (up to 10%) was annotated by a histopathologist for the presence of steatosis, melano-macrophage aggregates (MMAs), leucocyte infiltration and granulocytomas. These annotated areas were used to train ML models using random forest classifiers (steatosis, MMAs) and watershed algorithms (leucocyte infiltration, granulocytoma). The models were then applied to the whole images, and the presence of each lesion was quantified. Results were compared with traditional scoring of the same slides by two independent pathologists to obtain a percentage accuracy for each lesion. Preliminary results from a limited sample (40 images) indicate strong potential for this method. All lesions displayed high comparative accuracy ($\geq 80\%$) between traditional and ML-based results, with MMAs and granulocytomas reaching 95%, the common threshold for acceptability in medical science. Differences in accuracy between species were minimal for all lesions except leucocyte infiltration (*G. morhua*: 80%, *L. limanda*: 92%). As we continue this research, increasing the size of the training set and complexity of the models, we expect to improve accuracies and correlations between digital histopathology results and biological effects, and to expand this work to include more lesions and species. This work, combined with automated slide preparation and scanning, will allow rapid turnover of samples, supporting efficient monitoring and decision-making for the protection of marine and coastal ecosystems.

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4.11.P-We368 Challenges in Assessing Acute Toxicity of Environmental Samples in Brine Shrimp and Coral Toxicity Assays

Valentina Di Mauro¹, Lisa Stoetzel¹, Matthias Y. Kellermann¹, David Brefeld¹, Laura J. Fiegel¹, Samuel Nietzer¹, Mareen Moeller¹, Laura Lutjens², Sascha Pawlowski², Mechtild Petersen-Thiery² and Peter Schupp¹, (1)Environmental Biochemistry Group, Institute for Chemistry and Biology of the Marine Environment (ICBM), Carl von Ossietzky University of Oldenburg, Germany, (2)BASF SE, Germany
Coral reefs are well recognized as marine biodiversity hotspots providing various ecosystem services such as lively hoods via fisheries, tourism or coastal protection. However, they are increasingly threatened due to climate change and other anthropogenic stressors, which can enter coastal marine areas either directly (e.g. sunscreens) or through shipment, untreated wastewater and sedimentation (e.g. coatings, pesticides, heavy metals). Thus, the toxicity assessment of environmental samples towards reef building corals is needed to better understand its potential impact.

The testing of environmental samples on corals (here we use *Montipora digitata*) may bear some challenges, which are highlighted in this study. However, prior to the testing of the corals themselves, a range finder using brine shrimp *Artemia* sp. was carried out to address these challenges. Firstly, environmental samples, whether sediment, wastewater, or native water, contain complex mixtures of compounds, which, depending on the exposure regime, differ with respect to their toxicity. Secondly, seawater might interact with environmental samples, due to its composition of salts and trace elements, especially when seawater matrix compositions are changing (e.g., in salinity).

Initial results suggests that brine shrimp are equally sensitive as *M. digitata* when tested with individual substances. Therefore, this approach was also considered suitable for the testing of environmental samples. When using corals for the testing of environmental samples the following approaches may be adequate: use of solvents (in case of e.g. organic sediment extracts or pore water), use of double concentrated artificial sea water (in case of e.g. native water samples or sediment eluates).

This approach aims to support the advancement of consistent, coral-specific ecotoxicological testing of various environmental samples under ISO.

Disclaimer/Disclosure: The authors declare that this study received funding from BASF SE (Ludwigshafen, Germany) through the grant Toxicity tests with corals and coral reef communities, grant number 5551622859. Opinions expressed in the paper are those of the authors; the funder had no role in the study design, data collection, analysis or interpretation of the study findings.

4.11.P-We369 Comparing the Sensitivities of Oysters and Standard Aquatic Species to Pesticide Active Substances

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To support registration of active substances for plant protection products globally, testing on three taxonomic groups of aquatic organisms (fish, daphnia, algae) covering various trophic levels is generally required. However, there are differences in ecotoxicity data requirements based on geography. For example, a marine mollusk (e.g. oyster) test is currently a data requirement for pesticide registration in the United States, but the test guideline and endpoints are not aligned with the data requirements in the European Union. These discordances may lead to questions regarding the coverage of mollusks in the aquatic risk assessment. Therefore, the goal of this project was to compare sensitivities of oysters to standard aquatic test species to better understand if this taxon is covered in the existing risk assessment framework for pesticide active substances in Europe. Oyster acute toxicity data for pesticide active substances were obtained by searching US EPA's ECOTOX Knowledgebase and regulatory documents. Acute toxicity data for fish and invertebrates were then acquired from reliable regulatory sources for each compound to match with the oyster data, and sensitivity ratios (fish or invertebrate endpoint / oyster endpoint) were calculated. Results demonstrate that oysters are similarly or less sensitive (within a factor of 3) compared to the standard fish or invertebrate test species for ~80% of substances in this dataset. For ~20% of the substances, oysters were more sensitive than the standard acute fish and invertebrate test species. However, considering the standard assessment factor of 100, these preliminary results suggest that oysters would be covered in the European aquatic risk assessment framework. A more detailed assessment of the compiled dataset is currently being conducted to look for additional trends. For instance, the substances where oysters have clearly lower endpoints than those obtained from fish or invertebrate tests will be investigated in more detail. Overall, this data analysis will help to better inform regulatory authorities on how to address potential risks to taxa currently not covered in risk assessments.

4.12 Soil Environmental Risk Assessment: Navigating Changing Regulatory Frameworks and Field-Realistic Predictions of Contaminants, Mixtures, and Stressors in Europe and Beyond

4.12.T-01 MICROSIL – Proposal for a Refined Risk Assessment Scheme to Assess Effects on Function and Structure of Soil Microbial Communities

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The MICROSIL project identified meaningful endpoints for assessing effects on microorganisms exposed to chemicals in agricultural soils. Appropriate test methods were identified based on a literature search in combination with an evaluating concept. Afterwards, the sensitivity of the five identified, most suitable methods ISO 15685 (potential nitrification), MicroRespTM (substrate induced respiration), ISO 20130 (enzymatic activity), ISO 10832 (spore germination of arbuscular mycorrhiza fungi (AMF)) and the fingerprinting method ARISA was observed in three soils by using six test substances.

The nitrogen transformation test (OECD 216), was often less sensitive than other endpoints and showed deviations of up to factor 100 towards other endpoints.

Microbial respiration (MicroRespTM) and potential nitrification (ISO 15685) were less sensitive than other functional endpoints (N-transformation and enzymatic activity). Enzymatic activity (ISO 20130) presented the most sensitive endpoints. Thus, it is proposed to supplement N-Transformation by enzymatic activity (ISO 20130) in the first tier risk assessment.

The ARISA data evaluated via ordination plots revealed the same low endpoints as ISO 20130, but was less often the only approach resulting in the lowest observed effect value. ARISA has shown shifts in community structure (relative abundances) raised by chemical exposure and thus, could be useful in the lower tier. However, further research is needed to better understand the relevance of deviations from controls.

The spore germination of AMF (ISO 10832) was more sensitive than OECD 216, but less sensitive than ISO 20130. Thus, a test with AMF is also suggested for lower tier testing. The results of the project indicate that the ISO 10832 has to be revised to allow testing of AMF in natural soils. Soil type affects the results of the tests. LUFA 2.1 was not always the soil resulting in the lowest endpoints.

In the actual risk assessment for PPP, the soil microbial community and provided ecosystem services are considered to be not at risk, if effects of the maximum predicted environmental concentration on N-transformation (OECD 216) are not larger than 25 %, latest 100 days after application. Based on the results obtained here and considering the recommendations of EFSA (2017), it is recommended to supplement the first tier risk assessment by an additional test on bacterial function (ISO 20130, enzymatic activity) and a test covering effects on AMF.

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4.12.T-02 Effects of Realistic Pesticide Mixtures on Springtails, Terrestrial Plants, and Soil Microbes – An Overview

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The application of multiple pesticides over the decades has resulted in their long-term presence as complex environmental mixtures. This work aimed to assess the toxicity of realistic pesticide mixtures from 11 case study sites (CSS) to 3 different trophic levels (soil invertebrate, terrestrial plant, and soil microbes). The CSS were located in 10 European countries (Portugal (PT), Spain (SP), France (FR), Switzerland (CH), Italy (IT), Croatia (HR), Slovenia (SI), Czech Republic (CZ), The Netherlands (NL), Denmark (DK)), and Argentina (AR). The mixtures were composed of 5 pesticides (as active substances or metabolites), chosen based on their occurrence in soils from the CSS and their risk to the different groups. Three concentrations were used: median measured environmental concentration (MEC), predicted environmental concentration (PEC), and 5 times PEC (5PEC). A reproduction test was conducted with the springtail *Folsomia candida*, following the OECD guideline 232. Root growth inhibition test and seedling emergence and growth test were conducted based on the ISO 11269-1 and OECD 208 guidelines, respectively, with one monocotyledonous (*Triticum aestivum*) and one dicotyledonous species (*Lactuca sativa*). The microbial activity was assessed as basal and substrate induced respiration for C mineralization, and as potential ammonium oxidation and potential ammonification for N mineralization, based on the OECD guidelines 216 and 217. A significant decrease in springtail reproduction was observed in 4 CSS (ES/IT, PT, FR) at PEC. The insecticide chlorantraniliprole was present in these mixtures. Terrestrial plants were very sensitive to the exposure of pesticide mixtures in most CSS, with significant effects on seedling biomass and root growth at MEC. These results indicate that the presence of multiple pesticide residues in soil is an important route of exposure to terrestrial plants. The effects on C and N mineralization occurred at PEC and 5PEC levels in PT, SP, FR, CH, IT, CZ, and NL. Although the effects on C and N mineralization persisted up to 56 d in the most toxic CSS (PT and CZ), the microorganisms recovered after 84 d. The important outcomes from this work are: (1) effects were observed at realistic exposure concentrations (MEC and PEC); (2) one or two pesticides drove toxic effects in the mixture, for which their toxicity were known, and therefore these results were expected; and (3) in some cases, however, a mixture synergistic effect was suggested.

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4.12.T-03 Assessing the Risk of Tank Mixtures to In-Soil Organisms: From Single to Sequential Applications

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The current ecological risk assessment (ERA) framework in the EU inadequately addresses the risks associated with the simultaneous or sequential application of tank mixtures (TMs) of plant protection products (PPPs) to in-soil organisms. This gap, highlighted in EFSA's 2017 scientific opinion, is further emphasized by the EU's Farm to Fork Strategy. This study evaluated the risks posed by single and sequential applications of four TMs, used in soybean plantations, on three non-target in-soil organisms: *Eisenia andrei*, *Folsomia candida*, and *Gaeolaelaps aculeifer*. The TMs consisted of 12 PPPs (15 a.s.), an adjuvant, a pH regulator, and a fertilizer, differing in components and formulation. Chronic tests followed ISO and OECD guidelines using a natural sandy-loam soil from southern Portugal. Single applications tested increasing TM concentrations, while sequential applications involved applying one TM followed by another after 14 days. Risk was assessed using the exposure-toxicity ratio (ETR), with PEC values calculated via concentration addition model (5 cm soil depth, no interception) and pesticide degradation

estimated using first-order kinetics. NOEC and EC10 endpoints were used as endpoints of toxicity. Results revealed that ETR values varied across species, application scenarios, and endpoints. NOEC-based ETR indicated similar risks for *F. candida* and *G. aculeifer* in single and sequential applications. TM1 risks were PEC-driven, while TM3 and TM4 were toxicity-driven. Synergistic effects were observed for TM1+TM2, but antagonistic effects emerged in more complex mixtures (TM1+TM2+TM3 and TM1+TM2+TM3+TM4). EC10-based ETR showed increased risks in sequential applications for *E. andrei* and *G. aculeifer*, especially with TM1+TM2+TM3. TM4 reduced risks, resembling single application patterns. This study highlights the complexity of assessing real pesticide mixtures due to synergistic and antagonistic interactions and underscores the need for advanced ERA approaches. The findings emphasize the importance of species-specific responses and endpoint selection (e.g., NOEC vs. EC10) in developing comprehensive EU risk frameworks to protect soil biodiversity.

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4.12.T-04 Next Generation Soil Risk Assessment: Application of the FORESEE Earthworm Model for Europe

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Soil organisms, such as earthworms play a crucial role in the soil ecosystem and their potential risks due to pesticide exposure are assessed within the environmental risk assessment. Thus, the FORESEE model was developed, which is a mechanistic modular earthworm effect model, simulating earthworm behaviour to determine the effective exposure experienced in agricultural soils. The model includes the three major ecotypes of epigeic, endogeic, and anecic species and enables the extrapolation of lethal, sublethal and behavior effects from individual-level to population-level. It does that by considering the movement of the species which depends, just like the fate of chemicals, significantly on environmental parameters, such as moisture and soil conditions.

The model includes four modules: the environment, movement, toxicity, and the population module. Each module was parameterized and validated independently but interacts with each other in the overall framework. While the population level response can be predicted from behavior, sublethal and lethal effects on individual level, the FORESEE framework can be combined with field studies to prove the predictive power of the model. In the next step, the model can be used to extrapolate the results of the field study to other situations, e.g., soil and weather in different zones or different exposure situations as time or number of applications.

For this presentation, we will apply the parametrized FORESEE model that was shown to be able to predict the effects of Carbendazim on earthworms with different burrowing behaviour in a two-year field study. We will use the model to create in-silico field studies and estimate the impact of different application scenarios, such as timing, frequency, and crop stage, as well as environmental conditions from the three regulatory zones of Europe.

In conclusion, the FORESEE model can enhance our understanding of the effects of environmental fluctuations on earthworm populations and potentially support the design of earthworm field studies. The model can be used to assess the impact of different application rates, frequencies, and timings, different crops, as well as climates in other regulatory zones, on the long-term population responses of earthworms. Thereby, the model reduces uncertainty and increases the predictability of the risk assessment framework.

4.12.T-05 National Approaches to European Soil Threshold Values: A Review Towards a Degree of Harmonization

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Despite legislative initiatives dating back to 2006, there is currently no unified EU soil legislation in place. In the light of these earlier legislative efforts, an elaborate comparative analysis of several EU countries had been made on risk assessment frameworks and approaches to set Soil Threshold Values (STVs) for relevant pollutants. Within the context of the newly proposed Soil Monitoring and Resilience Directive (SMRD), the Horizon Europe project ARAGORN (Achieving Remediation And Governing Restoration of contaminated soils Now) initiated a new effort to compare STVs across Europe.

For all 31 countries in scope we identified relevant legal sources through national and international online public repositories. Identified legal and quasi-legal texts related to soil contamination were methodically searched and STVs extracted. Our findings have been validated as much as possible by national experts from the ARAGORN and COMMON FORUM networks.

The main finding of this comparison is that STVs are poorly harmonised across Europe. In our investigation of national legislations, we identified STVs for 26 out of the 31 countries. For five countries, no STVs were encountered in the national legislations, either because such values may not be established in these jurisdictions or because another (e.g. case-by-case) risk assessment approach is used. The median number of legislative entries (i.e. substance, sum parameter or oxidation state for metals) per country was 49, with a maximum of 130. Moreover, some countries developed different STVs for different land uses (up to more than 5 classes) while other countries apply uniform values irrespective of land use. Similarly, distinctions based on soil type (clay, loam, sand) or characteristics (pH, organic carbon content, water permeability) are applied in certain national frameworks, though these criteria are less common compared to land use. While not a primary objective of this study, STV derivation methods as well as the application of STVs within the national risk assessment frameworks showed considerable differences among countries as well.

It is concluded that the lack of harmonised STVs approach is potentially an impediment for effective soil management and remediation of contaminated sites. It is therefore suggested that the SMRD, currently under discussion, outlines a trajectory to a harmonised set of best practices for STV derivation and application in risk assessment.

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4.12.P Soil Environmental Risk Assessment: Navigating Changing Regulatory Frameworks and Field-Realistic Predictions of Contaminants, Mixtures, and Stressors in Europe and Beyond

4.12.P-We370 In-field Recolonisation Rate of Soil Fauna after Precision Application with High Impact on Sub-population

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The incorporation of precision application of crop protection products is part of the EU Green Deal ambition to reduce pesticide use by 2030. Digital farming solutions can help farmers apply pesticides precisely where and when they are needed. Spot treatment of weeds with herbicides, for example, will in many agricultural fields strongly reduce the total amount of applied herbicide compared to conventional broadcast application. However, there is a surprisingly extensive lack of knowledge about the realistic reduction of the environmental impact of precision applications, and how these impacts can be assessed in the current risk assessment schemes. If a full field pesticide application rate exceeds the chronic no effect level for a small set of soil species (incl. a safety factor), an unacceptable risk is concluded because the recovery potential of soil organisms is hampered by their relatively slow migration rate. Population recovery can be demonstrated in higher tier assessments by full scale field studies. Spot treatment of active substances (with raised concern for impact on soil organisms), however, would leave large portions of the agricultural field untreated, and the unaffected subpopulation in these parts may actively repopulate the impacted spots via migration. In order to account for this higher tier recovery process in specific precision application GAP scenarios, the recolonisation rate potential via migration needs to be assessed. The current study assessed the recolonisation rate of earthworms under field conditions in two experimental set-ups during 3 months, in either a 6 meter container (sieved soil, free of worms, no lasting impact), or high impact hot water injection on 5 x 5 meter plots within an excessively managed meadow (>90% impact on subpopulation, no expected lasting impact). Worm traps were monitored for occurrence of adult and juvenile earthworms biweekly, including total biomass. Once 2 or more replicate traps per sampling distance contained worms, this distance was considered recolonized. The linear movement test showed recolonization over 1.5 m until 6 weeks, and full recolonization over 6 m after 8 weeks. The field plots showed worm recolonization after 4 weeks in most of the 1 m and 2 m zones, and the central 2.5 m zone after 6 weeks. This provides more detail in migration capacity than literature values of 5-15 m/y. Similar set-up could examine migration capacity for other in-soil species.

4.12.P-We371 Analytics in Soil Laboratory Studies – Technical Challenges and Implications for Soil Risk Assessment of Plant Protection Products

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The Tier 1 risk assessment of plant protection products for soil organisms is conducted by comparing laboratory endpoints, e.g. no-observed effect concentration (NOEC) based on reproduction, growth, or mortality, with the predicted environmental concentrations in soil (PECsoil). So far, the ecotoxicological endpoints are expressed as nominal, initial concentrations, and are compared with the maximum, initial

PECsoil values. However, according to the Central Zone Manual analytical verification of the concentrations in the ecotoxicological laboratory tests over time are requested in case the DT90 of the compound is lower than the exposure period in the test. Time-weighted average (twa) or mean-measured endpoints shall be calculated in case the measured values do not reach 80% of the desired values. However, degradation of substances in soil is a normal phenomenon and cannot be avoided in most of the cases. Hence, a proper description of the real exposure over time in the test system can be useful for the risk assessment and may support decision making. However, many aspects remain unclear in this context and need clarity: More technical guidance is needed on the design of future laboratory studies that include analytical measurements: i.e., number of additional replicates for destructive samplings, potential introduction of soil organisms in these replicates, analytical methods. Further, the manual does not state how existing studies will be evaluated in risk assessment, e.g. EU-agreed endpoints or product endpoints. From the published manual it remains unclear how the new endpoints should be used in risk assessment. A case study is presented that shows the outcome of the Tier 1 risk assessment for two compounds with identical toxicity (nominal NOEC) with same application rate and crop stage. When considering twa NOEC and initial PECsoil values the persistent compound clearly passes the critical trigger value of 5, whereas the fast-degrading compound fails the risk assessment. However, fast degradation of compounds is per se a favorable substance property from an environmental perspective and a higher conservativeness at Tier 1 for fast degrading compounds is not justified. Hence, it is strongly recommended to consider PE Ctwa in the risk assessment in case the endpoints are expressed as twa or mean-measured endpoints.

4.12.P-We372 Central Zone Requirement for Analytical Measurements in Soil Organism Ecotoxicology Studies: What is the Impact?

Christopher Taylor¹ and **Jennifer Duncan²**, (1)*Consultant Ecotoxicologist, United Kingdom*, (2)*Marketing, TSG Consulting, Knaresborough, United Kingdom*

In addition to the approval and renewal of pesticide active substances at an EU level, Plant Protection Products (PPP) containing these substances must be authorised in EU Member States before they can be sold and used. These authorisations are often considered via a Zonal evaluation process, and each Zone may introduce additional or deviating approaches to standard EU-wide data requirements or risk assessment methods.

In August of 2023 the Central Zone published an update to their Working document on Risk Assessment of Plant Protection Products in the Central Zone. A significant addition to this Working Document is the requirement for ecotoxicology studies with soil organisms to include analytical verification of exposure throughout a study's duration, should the tested substance be considered as unstable defined as having a laboratory 90% degradation time (DT90) in soil of less than the duration of the ecotoxicology study. This represents a requirement not historically conducted for such studies and may therefore create a significant need to repeat studies to support future registration of PPPs in Central Zone Member States.

The aim of this presented work is to establish the impact of this new Central Zone requirement in terms of what proportion of pesticide active substances may trigger the need for analytical verification in one or more of the soil organism studies which are, in most cases, a standard regulatory data requirement for PPPs. Active substances making up the so-called AIR5 renewal list were investigated and their currently defined laboratory DT90 values were collected from publicly available EU lists of endpoints. These substance DT90 values were then compared to the in-life duration (according to current OECD test guideline methodologies) of each of the three soil organism study types included in the data requirements under Regulation (EU) No. 1107/2009.

The results of this work are presented as a proportion of the investigated active substances which would require analytical verification in at least one of the standard soil organism studies, as well as proportions which would require each individual study type to include concentration analysis. The outcome of this work illustrates the impact of this Central Zone requirement to conduct new soil organism studies (to include analytical verification of achieved exposure concentrations) to meet the requirements for future PPP registration in Central Zone Member States.

4.12.P-We373 Analytics in Soil Ecotoxicological Studies: Challenges and Approaches from a Regulatory, Biological and Analytical Perspective

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For the assessment of plant protection products in the European Central Zone, analytical measurements are requested for soil organism studies for some cases. Substances where the DT90 does not cover the exposure phase of the laboratory Tier 1 tests are considered as unstable and analytical dose verification must be performed at least at the start, middle and end of a study, in general.

Currently, there is only very limited guidance available and many challenges. The time points for soil samplings depend on the substance degradability and the test duration differs from organism to organism. Consequently, each product or compound needs a tailored study design to include the analytical verification. The selection of timepoints are a challenge, as the DT90 and DT50 derived in natural soils could differ from that in OECD artificial soil. For the earthworm testing, the test organism biology might play a role for biodegradation patterns in comparison to plain soil, as earthworms likely increase the microbiological soil activity by incorporating their feed cow dung into the soil.

In a fictive scenario, we will present possible experimental designs for *Folsomia candida* and *Eisenia fetida* using destructive sampling with additional replicates for the soil analytics. The possible challenges and approaches for soil extraction and soil analysis using LC-MS/MS will be presented.

Moreover, we will compare fictive geomeans of a fast, medium and slowly degrading active on the basis of DT90 and DT50 values to evaluate the impact of using geomean endpoints derived by analytical assessment in the Risk Assessment.

4.12.P-We374 Rethinking Soil Safety: How the 2023 Central Zone Guidance Change the Risk Assessment for Rapidly Degrading Active Substances – A Practical Case Study

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Soil risk assessment for plant protection products in the EU traditionally follows the Terrestrial Guidance Document (SANCO/10329/2002), using a toxicity exposure ratio (TER) that compares toxicity endpoints from laboratory studies to modeled maximum predicted soil concentrations (PECmax). Historically, toxicity endpoints have been derived from nominal concentrations.

However, the recent 2023 Working Document on Risk Assessment of Plant Protection Products in the Central Zone introduces a new methodology for active substances with DT90 values below the soil toxicity study duration for earthworms, springtails and soil mites (56, 28, or 14 days). For such active substances, analytical verification of soil concentration is required according to this methodology, in contrast with relevant test guidelines in which analytical work is not required, and when concentrations fall below 80% of nominal, study endpoints should be based on time-weighted average (TWA) or geometric mean measured concentrations. TER calculations would then rely on estimated worst-case PECmax peak values (not considering the rapid degradability in soil for a substance) and measured endpoints over a long period of time, leading to unrealistic risk assessment outcomes. Without available intermediate tier testing options, this change in the regulatory framework may lead to an increase in highly complex soil organism field studies.

This poster aims to analyse different risk assessment approaches, considering soil PECmax and PECTWA values, the sampling time points and the impact on TER calculations. For this purpose, a comparison between a set of ecotoxicological soil studies carried out according to the SANCO (2002) approach with endpoints from tests without analytical methods with the updated 2023 Central Zone approach - where analytically verified endpoints were derived - is presented here. We highlight the impacts and limitations of this new approach and discuss potential future developments for a more pragmatic soil risk assessment framework.

4.12.P-We375 MICROSOIL - Degradation Performance of Microbial Communities in Agricultural Soils after Multiple Exposure of Pesticide Active Substances

Kerstin Derz¹, Karsten Schlich¹, Dimitrios Alexander Skodras¹, Udo Hommen¹, Bjoern Scholz-Starke², Anna Pissarello³, Frank Zielinski³, Jens Schonfeld³, Pia Kotschik³ and Silvia Pieper³, (1)Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany, (2)darwin statistics, Germany, (4)German Environment Agency (UBA), Germany

The degradability of active substances used in chemicals like plant protection products is currently estimated in laboratory tests (usually according to OECD Test Guideline 307) with standardised soils and one maximum application rate. However, agricultural used soils are exposed to multiple chemicals. Besides plant protection products, also pharmaceuticals, biocides as well as industrial chemicals enter soils through sewage sludge, fertilizers or slurry. Within one growing season, several plant protection products which may include more than one active ingredient and multiple application schemes are used, but the occurrence of multiple residues in agricultural used soils is not reflected by the current risk assessment schemes for plant protection products or other chemicals.

Within the MICROSOIL project, commissioned from the Germany Federal Environmental Agency (UBA), the correct assessment of the performance and threats of microorganisms in agricultural soils was investigated. One of four work packages analysed whether multiple applications of one substance, binary mixtures and background contamination affect the degradation time in soil. Two representative active

substances, pyraclostrobin and ethofumesate, and two representative standard soils, RefeSol 02 -A and LUFA 2.1, were used for this purpose. The results show, that multiple applications of the same substance as well as the presence of another substance in soil may have both positive and negative effects on the degradation rate of the test substances used in this project. However, a negative impact on the degradation rate was shown, for instance, for ethofumesate when applied to a soil where a second substance (here: pyraclostrobin) was already present. The results indicate that further research on multiple exposure is necessary to consider more realistic use patterns and agricultural practices.

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4.12.P-We376 MICROSOIL - Antimicrobial susceptibility testing to evaluate minimum inhibitory concentration values of relevant antibiotics in soil bacteria

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Within the MICROSOIL project minimum inhibitory concentrations (MIC) of for four different soil bacterial species have been determined for four antibiotics and for two other substances (heavy metal salt and detergent) were investigated in relation to soil microorganisms. While the EUCAST database provides MIC data for clinical bacteria, similar information for soil bacteria is lacking. By evaluating whether soil bacteria are more sensitive to antibiotics than clinical strains, this study provides essential data for environmental risk assessments.

Strains were chosen based on their ecological relevance in soil, maximizing diversity among the selected organisms, and the availability of genomic data. Four strains were selected, with known genomic information: *Arthrobacter* sp., *Lactobacillus plantarum*, *Pseudomonas gessardii*, and *Acidovorax facilis*. MICs were determined using the broth microdilution method following EUCAST protocols. For each substance (colistin sulfate, neomycin sulfate, chlortetracycline hydrochloride, tiamulin hydrogen fumarate, copper sulfate, and TWEEN 20.), eight concentrations were tested with three controls included: inoculation control, substance control, and media control. The variability in MICs between strains spanned up to two orders of magnitude; *Acidovorax facilis* showed the lowest MICs for two of the antibiotics tested, while *Arthrobacter* sp. was never the most sensitive strain. The lowest MIC recorded was 0.06 mg/L for chlortetracycline hydrochloride. In comparison with MICs derived for clinical strains (available from EUCAST database) no systematic different sensitivity of the soil strains tested here was found. For the four antibiotics tested, a predicted environmental concentration for soil based on the intended use of this substance was only available for Tiamulin. The risk quotient (RQ) based on the minimum MIC and without using an assessment factor was >100 and larger than the RQ based on the standard ecotoxicological tests, suggest that MIC could be a relevant endpoint in the risk assessment of antibiotics.

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4.12.P-We377 How to Include Effects on Soil Functioning in Soil ERA? An Ecological Perspective

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Soil contamination of any kind (metals, organics, plastics, nutrients) may disrupt soil microorganisms with important roles in carbon (C) and nitrogen (N) cycling. Effects of contaminants on soils has traditionally been most focused on its visible inhabitants, mainly plants and wildlife, and biased towards economically important crops. The effect of contaminants on soil microbial communities and their activity has been largely neglected, or overly simplified. Often, effects on soil microorganisms are tested with short-term tests under laboratory conditions. However, how the functioning of microorganisms are affected in field soils is unclear, which may lead to a mismatch between soil ERA and field effects.

Microbial parameters with a direct link to soil C and N cycling such as microbial respiration, biomass, N process rates and enzyme activities are often proposed to assess changes in soil quality and rely on a large availability of well-established methods. Microorganisms are known to response rapidly to environmental changes (hours days) as they have intimate relationships with their surroundings due to their relatively high surface-to-volume ratios. However, the variable intensity and direction of response make their implementation and interpretation in soil risk assessment challenging. Instead, a combined approach that includes plant and soil parameters provides a more integrated view on potential ecosystem changes.

To demonstrate this approach, various studies were undertaken to assess the effects on C and N cycling in contaminated soils under realistic (field) conditions. A variety of microbial responses, such as in situ microbial soil respiration (BR, SIR), biomass, and N cycling microbial guilds were measured in two historically contaminated sites and two outdoor field trials. The microbial responses were linked to slower responding plant and soil variables and stable isotopic content $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

The ecosystem effects in the two field soils, and soil specificity of microbial responses in the N cycle, support the view that soil contamination can have long-lasting effects. However, different intensities and the lack of connection with bioavailable fractions of metals, stress the need for site-specific approaches.

4.12.P-We378 ERAMYC: Results of a Ring Test for Standardization of Arbuscular Mycorrhizal Fungi Pre-Symbiotic and Symbiotic Phases Tests for Environmental Risk Assessment Schemes

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The presence of arbuscular mycorrhizal fungi (AMF) in terrestrial ecosystems is essential due to the benefits these organisms provide to their host plant and to several key ecosystem services.

Ecological relevance and sensitivity of AMF to contaminants led the European Food Safety Authority (EFSA) to identify AMF as a potential group of non-target in-soil organisms to be included in risk assessment of plant protection products (PPPs). To date, there are no standardized OECD Test Guidelines for the performance of tests with AMF, but two standardized methods are available, the ISO/TS 10832:2009 for pre-symbiotic testing and the AFNOR, FD X 31-205-2, for symbiotic testing.

The ERAMYC project aims to fill some of the gaps identified by EFSA by improving the existing ISO document for pre-symbiotic testing and by developing and standardizing a laboratory method for symbiotic testing. A ring test was conducted to assess the feasibility and reproducibility of these methodologies with the final objective of developing an OECD international standard for AMF pre-symbiotic and symbiotic testing.

A methodology to assess the effects of the test substances on the AMF-plant symbiosis was developed and the method described in the ISO protocol for pre-symbiotic testing was adapted and refined with new methodological steps and different test conditions. The ring test for both methods was carried out using the AMF *Rhizophagus irregularis*, the host plant *Allium ampeloprasum*, OECD 2.5% soil and the test substances azoxystrobin and fluazinam.

Results of the symbiotic phase tests showed some variability and so far it was not possible to find a suitable reference substance. Toxicity observed in the pre-symbiotic phase tests tended to be lower than that observed in the symbiotic phase tests.

The variability of data obtained, especially in the AMF symbiotic phase testing, and the difficulty in finding a suitable reference substance may hamper the standardization of the method. On the other hand, the toxic effects of the test substances to AMF observed in the ring test tended to be in the same order of magnitude or higher than the toxicity data from literature for non-target standard in-soil species, which suggests that the AMF communities may, in some cases, be insufficiently covered by the standard species currently considered in the ERA of PPPs in European Union. However, further tests are needed with a larger set of test substances to substantiate this assumption.

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4.12.P-We379 Independent Yet Complex: Decoding Soil Moisture and Pesticide Interactions in Toxicity Tests with *Folsomia candida* (Collembola)

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Soil humidity conditions are crucial factors in the life of soil invertebrates, but it can also significantly influence the toxicity of pesticides which may be taken up through the soil pore water. Yet its role remains underexplored in ecotoxicology. This study therefore evaluated the impact of soil moisture content on the toxicity of the insecticide teflubenzuron to the collembolan *Folsomia candida* using OECD 232

reproduction tests. We tested six concentrations of teflubenzuron plus two controls, combined with eight levels of soil moisture corresponding with soil water potentials between 1.8 and 721 -kPa. This broad range covers optimal, extreme drought, and wet conditions, providing a comprehensive overview of soil moisture interaction with pesticides. After 28 days of exposure, we characterized the interaction between soil moisture content and teflubenzuron effects on adult survival, final body mass of adults and reproduction, using the MIXTOX tool. The concentration addition model showed dose-ratio dependent antagonism for reproduction, but no deviations from additivity for body mass. The independent addition model best explained the combined effects of soil moisture and teflubenzuron on springtail reproduction and biomass. This indicates that neither synergistic or antagonistic interactions, nor dose-level or dose-ratio dependence deviations were found. However, survival patterns were more complex with higher survival rates in dry soils at high teflubenzuron concentrations compared to moist soils. Further analysis confirmed that teflubenzuron accumulation was greater in moist soils at high exposure levels, explaining the higher mortality at high soil moisture. This study thoroughly investigates how soil moisture content interacts with pesticides and provides added value to the standard toxicity test.

4.12.P-We380 Effects of Metal-Based fungicides on the Growth, Reproduction and Avoidance Behaviour of *Eisenia Andrei* Under Varying Temperatures and Moistures

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This research investigated the ecotoxicological effects of metal-based fungicides in the context of contemporary global climate change scenarios, specifically at temperatures of 20°C and 25°C, and moisture levels of 30% and 50%. The study focused on both single and binary mixtures of copper oxychloride (CuOx) at concentrations of 200, 500, and 1000 mg/kg, alongside mancozeb (MnZn) at 44, 850, and 1250 mg/kg. Key endpoints evaluated included mortality rates, biomass alterations, avoidance behaviour, and reproductive success, following standardized protocols established by ISO and OECD. The assessments of biomass and mortality were conducted over a 28-day period, succeeded by a 28-day reproduction assessment and a two-day avoidance behaviour evaluation. Across all temperature and moisture combinations, mortality rates in the exposed groups surpassed 10% solely in the CuOx1000 and CuOx1000+MnZn1250 mg/kg groups. Notably, at 20 °C and 30% moisture, the mortality rate exceeded 10% only in the CuOx500+MnZn850 mg/kg treatment. The relative growth rates in the CuOx and MnZn treatment groups exhibited a decline with increasing concentrations. In the CuOx, MnZn, and their binary mixtures under conditions of 20 °C at 30% moisture and 25 °C at 50%, avoidance behaviour remained above 80% throughout the exposure period, with exceptions noted for CuOx200 mg/kg, MnZn44 mg/kg, and CuOx200+MnZn44 mg/kg. The reproductive output of earthworms in all treatment groups was found to be concentration-dependent and influenced by varying temperature and soil moisture conditions. Notably, no juveniles or cocoons were produced in the CuOx1000 mg/kg treatment at 25°C and 30% moisture, suggesting that copper oxychloride may exhibit greater toxicity than mancozeb, particularly under drought conditions. This study concluded that variations in temperature and soil moisture significantly affect the ecotoxicity of CuOx and MnZn, indicating that climate change is likely to have profound implications for the ecological roles of metals in earthworm populations.

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4.12.P-We381 The Effect of Environmental Conditions on Heavy Metal Toxicity to Earthworm *Eisenia fetida* in Sewage Sludge-Amended Soil

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The amount of sewage sludge (SS) is continuously increasing, posing challenges for its utilization. SS is primarily applied in agriculture and landfiling, but its benefits for soil due to high organic matter and essential nutrients are countered by its pollutant content, including heavy metals (HM), organic pollutants and other toxic substances. Current SS application regulations only focus on HM and nutrient levels, overlooking risks to soil biota and ecosystem functions. Climate change alters the dynamics of pollutants, affecting their dispersion, bioavailability, and impact on soil organisms. Consequently, SS fertilization rates must be adjusted with ongoing climate change. Earthworms are widely recognized as model organisms for assessing soil toxicity due to their ecological significance, sensitivity to contaminants and reliability as indicators of soil health. Adverse impacts of SS on earthworms can impair ecosystem functions, leading to diminished soil fertility and biodiversity. Understanding how increasing temperature

and altered soil moisture will change SS impact on soil dwelling biota is critical for SS management and reuse in agriculture and forestry.

This study aimed to analyze the single and combined effects of air temperature (21 and 25°C) and soil water content (SWC optimal, reduced, and elevated) to earthworm *Eisenia fetida* in SS amended soil (0-50 t/ha). Growth and mortality were measured weekly, and oxidative stress was assessed at the end of the experiment using biochemical indicators such as superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA). The results showed that *E. fetida* is tolerant to low HM mixture pollution in SS-amended soil under changing climate conditions. Lowest SS doses (12.5 and 25 t/ha) promoted growth at optimal SWC, while the highest SS dose (50 t/ha) caused the biggest mortality in all analyzed SWC levels. The earthworm's antioxidant system, crucial for metal detoxification, played a key role in mitigating toxic effects. Elevated temperature (25°C) intensified the harmful impact of HM across all SWC levels. Controlling environmental conditions (temperature, substrate compatibility, etc.) and pollutant levels is crucial to soil organisms. This study provides insights into the interactions between climate factors, pollutant toxicity, and soil organisms, offering valuable implications for future bioremediation efforts and the use of SS as fertilizer.

4.12.P-We382 Transforming Planetary Regolith into Viable Substrates: An Ecotoxicological Approach

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Space agencies have been planning long-term missions to the Moon and Mars for many years. Currently, bioregenerative life support systems are used to provide food in space. Utilizing in situ resources and limiting terrestrial input into these systems would be ideal as it would be cost-effective. In situ resources, however, contain potentially harmful elements, such as heavy metals and fine particle matter. Overall, there is limited research on the toxicological profile of planetary regolith. Therefore, this study aims to assess the toxicity of the regolith using earthworms as bioindicators and turning the regolith simulants into soil that can support the growth of radishes. Lunar Highlands (LHS-1) and Martian Global (MGS-1) simulated regoliths were used alongside a control as earth soil. Growth, reproduction and avoidance assays with *Eisenia andrei* were carried out using the simulants at 100%, 80%, 60%, 40% and 20%. To transform the regolith, organic compost and *Eisenia andrei* were added to the soil to support the plant growth. The final concentrations of Earth, Lunar and Martian soils in the experimental setup were 70%, 70% and 50%, respectively. *Raphanus sativus* (radish) was grown in the soil mixtures over three cycles. On day 28, the plants were harvested and measured before being oven-dried. They were weighed, and the metal content in the leaves, radishes, soil and worms was determined using ICP-MS. Regarding the earthworm exposures, 100% LHS-1 and 100% and 80% MGS-1 resulted in total mortality. However, LHS-1 at 20%, 40%, 60%, and 80%, and MGS-1 at 20% showed minimal adverse effects in growth and reproduction assays. The earthworms did, though, avoid both regoliths at 60% and 80%. Finally, MGS-1 at 40% and 60% resulted in impaired growth, a lack of juveniles, and 60% led to eventual mortality. In the potting experiment, the radishes were successfully grown in the Lunar soil with biomass inhibition as low as 32%, and in the Martian soil, inhibition was as high as 80%. Whilst there were no significant differences across the three growth cycles, this study hypothesizes that there is heavy metal transfer to the plants and earthworms that could make them harmful for consumption. The findings of this research could contribute valuable insights into sustainable agricultural practices for future space exploration and colonization.

4.12.P-We383 Approaches to Better Monitor the Effects of PPP on Soil Mesofauna: A Regulatory Perspective

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For the authorization of plant protection products (PPP) in the context of the Regulation (EC) No 1107/2009, an a priori regulatory risk assessment includes a risk characterization for the soil compartment and soil non-target organisms such as earthworms or collembola and acari. For these organisms, data requirements set by Reg. (EU) No 283 and 284/2013 are chronic laboratory studies. Following the European guidance for terrestrial ecotoxicology (EC, 2002) tiered approach, unacceptable adverse effects identified with these laboratory data would likely trigger the need for more complex higher-tier studies. Classically, field study are performed and used to refine the risk assessment, accordingly. While a guideline is available to conduct earthworm field studies, no such guideline is yet available for soil mesofauna. This lack of clear methodology introduces numerous shortcomings related to study design, sampling methods and species identification as mesofauna field studies are mainly performed following recommendations set for other soil-dwelling organisms (i.e. earthworms) but not exclusively (i.e. non-

target arthropods). To date, field studies focused almost exclusively on overall abundance variation related to PPP application despite the fact that soil mesofauna is known to be involved in primordial ecosystemic services in soil (i.e. soil aggregation, organic matter degradation, etc.). To overcome these issues, it appears interesting to develop/test existing approaches already available for soil quality evaluation. For instance, despite the fact that abundance and identification to the species level are commonly performed in these field studies, nothing is proposed to calculate diversity indices to obtain information about community dynamics after application of PPPs. In addition, trait-based approaches recently developed to study soil invertebrates responses to environmental changes may be regarded as promising a tool. More precisely, functional traits analysis have already showed good results in the context of contaminants exposition (Metallic trace elements, PPP, etc.).

The aim of this poster is to identify and discuss existing approaches that would be likely useful for mesofauna field studies in the frame of Reg. (EC) N°1107/2009. Pro and cons would be listed and relevance and reliability of each approach will be discussed.

4.12.P-We384 Normal Operating Range of Earthworms in European Agricultural Fields

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Normal operating range (NOR) is used as a measure of natural variability of ecological quantities, but its exact definition and method of calculation depends on the intended use. It has been proposed to use NOR to define an ecologically relevant level of effects of plant protection products on populations of non-target organisms, both when effects are quantified with field studies as well as with population models. In this contribution, we demonstrate how a NOR for earthworms can be calculated based on systematic spatial variability of abundance in untreated control data from field effect studies, using a generalized linear random effects model. We quantify NORs for three species of earthworms common in European agricultural fields, *Lumbricus terrestris*, *Aporrectodea caliginosa* and *Aporrectodea rosea*, using data from 27 field studies conducted in Denmark, Germany, Spain and UK between 1997 and 2021. At the scale of a field study, we determined relative spatial 90% NORs of 87% to 115% for *L. terrestris*, 80% to 125% for *A. caliginosa*, and 64% to 157% for *A. rosea*. While there were indications of seasonal differences in abundance, the relative spatial variability was well described by a single range across all seasons. Remaining variability fitted well to the Poisson distribution for *L. terrestris*, while data for *A. caliginosa* and *A. rosea* displayed overdispersion and were better described using a negative binomial distribution.

4.12.P-We385 Recovery of Earthworm Populations in Field Test Plots Through Migration?

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Earthworm field tests are used as highest tier in the risk assessment for in-soil organisms. Differences in earthworm populations (population size and composition) of test item treated and untreated field plots are assessed at different timings after application of the test item, thus covering short-term effects (1 month after application) as well as medium to long-term effects (6 months, 12 months after application) including recovery.

If full recovery is observed after a one-year trial period, the risk is estimated to be low (De Jong et al., 2006; EFSA, 2017). The test (ISO guideline 11268-3, ISO 2014) is typically carried out on plots with a minimum size of 10x10 m that are separated from each other by untreated strips.

It is known that earthworms are capable of active dispersal (e.g. EFSA 2017, Grigoropoulou & Butt 2010, Emmerling & Strunk 2012) and it could thus be postulated that migration could lead to an overestimation of the recovery potential of earthworm populations when studying small experimental plots especially when the distance to potential source areas for migration (untreated areas) in relation to the potential dispersal rate is much smaller than in real world field situation with much larger distances to untreated areas (field margins etc.).

A series of field experiments was set up in order: (a) to estimate the contribution of migration from untreated areas to the recovery of strongly damaged earthworm populations and (b) to evaluate whether migration could be reduced by either setting up mechanical barriers at the plot borders or by separating the plots from untreated areas by strips of intensively cultivated soil. Additionally, data from >50 earthworm field studies following ISO 11268-3 with pairs of toxic standard and water-treated plots were evaluated. Results of these field experiments and data assessments will be presented and discussed.

4.12.P-We386 Comparison of Soil Ecotoxicity Results Using the TRIAD Method and USETox Ecotoxicity Assessment for Abandoned Mine Sites

Seung-Woo Jeong¹, Songhee Kim¹, Rabin K.C¹, Dajung Kim¹ and Youn-Joo An², (1)Kunsan National University, Republic of Korea, (2)Konkuk University, Republic of Korea

To assess the ecological risk of contaminated soils, the ISO has recently proposed the TRIAD method. This approach evaluates Integrated Risk (IR) of contaminated sites based on three Lines of Evidence (LOE): chemical assessment, ecotoxicological assessment, and ecological assessment. In contrast, USETox is an ecotoxicity impact assessment method used in Life Cycle Assessment (LCA) to evaluate the potential ecotoxic effects of pollutants released into specific regions. The fundamental difference between these two methods lies in their focus: while the TRIAD method provides a practical ecological risk assessment of already contaminated sites, the USETox method predicts potential ecotoxicity caused by pollutant emissions. This study compares the practical ecological risk assessment results from the TRIAD method with the potential ecotoxicity impacts calculated using USETox for two abandoned mine sites.

The TRIAD method indicated a "Moderate Risk" or higher for both sites, with minimal differences in ecological risks between them. However, the absolute ecotoxicity values derived from the USETox method were significantly higher. Despite these differences, the ranking of ecological severity between the two sites was consistent across both methods. The notable discrepancies in ecological risk outcomes between the two methods are attributed to differences in exposure scenarios and spatial coverage. The TRIAD method calculates risks based on direct exposure to contaminated soil within the site, while USETox employs predicted values derived from Species Sensitivity Distributions (SSD) and assumes uniform distribution of contaminants across the entire site. This study suggests that USETox could be effectively utilized for prioritizing ecological risk assessments and remediation efforts for contaminated sites. Further research on USETox adaptations to improve reliability for site-specific soil ecotoxicity evaluations is also recommended.

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4.12.P-We387 Evaluation of an AI-based Software System for Counting and Body Size

Measurement of the Springtail *Folsomia candida* in OECD 232 Reproduction Tests

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The springtail reproduction test (OECD 232, 2016) with *Folsomia candida* is an essential part of the test battery for environmental risk assessment of plant protection products in the soil compartment.

Even though the reproductive output for a valid test requires 100 juveniles per control replicate at the end of the 28-day test period only, the actual number of juveniles is usually much higher and can reach values well over 1000. After extraction from the test soil with dyed black water, the white coloured adult and juvenile springtails are floating on the water surface and can be counted either directly under the binocular or on the computer using photographs taken from the water surface. In either way, manual counting is very time consuming, laborious, and prone to error.

To facilitate, accelerate, and standardise the counting process, the software FOLSOMIACOUNTER was developed to enable automated juvenile counting from photographs of the water surface after extraction. Additionally, an AI-based functionality allows estimating the body size of the counted adult and juvenile springtails. This supports observers to look for sublethal effects other than reproduction. Over the past years, the AI of the software has been continuously trained and improved with photographs of different quality.

We compare the results on counting juveniles on photographs from the yearly toxic reference item studies from 2016 until 2024 on both methods: manual and software-based assessment. These photographs were chosen because they are not part of the continuous training process of the software. The results regarding the following dimensions are discussed on the poster: (a) the achieved time saving, (b) the counting accuracy, (c) the impact on the main ecotoxicological endpoint EC50, and (d) the potential of body size measurement as an additional sublethal parameter in this type of test.

4.12.P-We388 The FORESEE Earthworm Model – from Conceptualisation to Completion

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Soil risk assessment of plant protection products is based on a two-tiered approach, where if the Tier 1 is not passed, field studies can be conducted to demonstrate that soil organisms are not at long-term risk. Field studies are time- and labour-intensive, and can only represent a snapshot of conditions, i.e., the tested climatic conditions, soil properties, and species composition. Mechanistic population models are a promising tool to extrapolate from these studies to different populations and environmental conditions. For the past 6 years, we worked on an earthworm population model, namely the FORESEE, to establish such a tool. The model was setup through collaborative efforts of experts from many disciplines, reaching

from earthworm ecologists, fate and risk assessment experts to modellers, to ensure a scientifically sound model with the help of the combined expertise and broad stakeholder engagement. The model integrates state-of-the-art concepts, such as dynamic energy budget and toxicokinetic-toxicodynamic theory, in a modular approach to provide a generic model, which supports a quick and easy change of species, ecological type and environmental conditions. In this modular approach the different model assumptions are readily interchangeable, which makes it a flexible tool for intermediate and higher tier risk assessment applications.

To encourage and inspire the set-up of more such models and enhance acceptability of these for soil risk assessment, we present the time course and important milestones of our project from first ideas collected in a workshop in 2018 to the final model validated with a field study to be published in 2025.

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4.12.P-We389 Assessment of Surface Water and Soil Quality in the Vicinity of Cattle Farms Using an Ecotoxicological Approach

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Currently, more than 5.7 million livestock farms are counted in the EU. Annual manure production in the EU reaches around 1.4 billion tonnes of which 75% derives from cattle. Manures are commonly applied to agricultural land for fertilization and represents a major route for the spread of nutrients and veterinary antibiotics in the environment. The main purpose of this study was to analyse soil and water quality in the vicinity of cattle farms of different size (500-1000 and >1000 livestock units (LU) through chemical and ecotoxicological parameters. The toxicity of surface water and soil leachates were assessed with *Lemna minor* and *Daphnia magna* bioassays. Results indicated that environmental surface water and soil samples exhibited inhibitory effect on the growth of *Lemna minor* and *D. magna* reproduction. Impaired *L. minor* growth and *D. magna* reproduction could be linked to antibiotic concentration in the samples.

4.12.P-We390 Promoting Sustainable Agriculture with Entomo-Fertilisers from Black Soldier Fly: Nutrient Leaching and Ecotoxicity Assessment of Leachates

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The overuse of chemical fertilizers in agriculture has significantly degraded soil quality, reducing biodiversity and harming ecosystems health. Nitrate leaching is a particular concern and occurs when rain and irrigation occur through soil layers, eventually reaching and contaminating groundwater reservoirs. Organic fertilizers derived from insect waste bioconversion - entomofertilizers - have been explored to address this environmental issue as sustainable alternatives that may partially replace conventional fertilizers. Besides their slow nutrient release into the soil, entomofertilizers are generated through a circular production process that reuses waste organic materials, such as olive pomace, which would otherwise have toxic environmental effects. This study aims to evaluate the leaching behavior and environmental impacts of different entomofertilizers produced from Black soldier fly (BSF) bioconversion (cereal and olive pomace as a substrate) in the soil, compared to mineral synthetic fertilizer. The leaching experiment was performed similar to OECD guideline 312, with some adaptations. Fertilizers were incorporated into standard Lufa 2.2. soil at a depth of 10 cm at the top of the column. The soil columns water-holding capacity was adjusted to 60%. Six treatments with five replicates were tested: control (no amendment); soil amended with a synthetic fertilizer; BSF cereal frass, and BSF olive pomace frass. Both entomofertilizers were tested in powder and pellet form according to the commercial formulations. A simulated precipitation rate of 221.9 mm was applied over 28 days, representing a winter month in Aveiro, Portugal. Leachates were collected, quantified and frozen after every second precipitation day. At the end of the experiment, leachates were subsequently filtered and analyzed for nutrient characterization and acute ecotoxicological assays using *Raphidocelis subcapitata*, *Lemna minor* and *Daphnia magna* were performed. Nutrient content in both the top and bottom soil layers was also determined. Nutrient leaching was reduced under frass treatments compared to synthetic fertilizer, with lower nutrient loss to leachates as well. We also observed no ecotoxicological effects in the tested organisms exposed to the leachates. This study provides valuable insights into BSF-based fertilizers direct and indirect environmental impacts, supporting a shift toward more sustainable agriculture practices.

4.12.P-We391 Pesticide Residues in African Agricultural Soils

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According to Tang et al. (2021), 64% of global agricultural land is at risk of pesticide pollution by more than one active ingredient, and 31% is at high risk. Among the high-risk areas, about 34% are in high-biodiversity regions, 5% in water-scarce areas and 19% in low- and lower-middle-income nations. Validation of these figures by field measurements is particularly challenging. This is mostly because of the limited post-approval monitoring of pesticides, and lack of soil-specific protection instruments, in Africa, and in general, but also because of the high costs and analytical challenges of analyses of such a diverse group of contaminants.

There are a few studies on pesticide contamination on African soils, but of rather limited coverage (geographical area, pesticide residues), and high variability on field and analytical details, hampering an overview of contamination, and ultimately of soil health. Therefore, to provide a first assessment of pesticide contamination, over 300 of the Soils4Africa soil samples were tested for a wide spectrum of pesticide residues (n=165). The h2020 Soils4Africa project aims to provide an open-access soil information system populated with data on the basic soil properties of ~ 20,000 topsoil samples. Pesticide samples were collected arable systems, are assumed to cover a mixture of real-field conditions (crops, farming systems, and pesticide use regimes). The target list was put together based on residues found in literature, application records, and pre-screening of 10% of samples. Pesticide analyses were done via LC-MS/MS-based and GC-HRMS-based methods, according to the JRC LUCAS 2018, and EU-SPRINT 2021 SOPs. The analytical work is being finalised and will allow to make inferences on overall, country, crop, and pesticide-level; with the main indicators being: number contaminated/ clean samples; Residues found/not found; mixtures in soil, Number residues/sample, level residues/sample, Frequency detection of different pesticide residues. Inferences on levels/thresholds/hazard of pesticides will be also performed. Our results can ultimately help define soil and pesticide monitoring priorities/watch list, assess pesticide pressure on ecosystems and human health, and Support decision-making concerning pesticide use/approvals/transition.

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4.12.P-We392 Effects of Realistic Pesticides Mixtures on the Springtail *Folsomia candida*

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The application of multiple pesticides over the decades has resulted in their long-term presence as complex environmental mixtures in European agricultural soils. Recent publications have shown that European soils present high levels of some pesticides, which may represent a risk to terrestrial non-target species. Therefore, assessing the risks of pesticide mixtures is extremely important. This work aimed to assess the toxicity of 11 pesticide mixtures to springtails. For each mixture, 5 pesticides were selected based on their occurrence in soils from 11 case study sites (PT-Portugal, SP-Spain, FR-France, CH-Switzerland, IT-Italy, HR-Croatia, SI-Slovenia, CZ-Czech Republic, NL-The Netherlands, DK-Denmark, and AR-Argentina) and their risk quotient. The risk quotient was calculated using available data on their toxicity to soil invertebrates and predicted environmental concentrations (PEC) retrieved from EFSA reports. Three concentrations were chosen: the median measured environmental concentration in soils (MEC), the predicted environmental concentration (PEC), and 5 times PEC values (5PEC) of the selected pesticides. Reproduction tests with the springtail *Folsomia candida* were conducted in agricultural soil, following the standard guidelines. Data was analyzed by One-way ANOVA, followed by Dunnett's post hoc test. Effects on springtail survival, size, and/or reproduction were observed in 5 mixtures. In PT and FR mixtures, effects on survival and reproduction were observed at PEC and 5PEC. In SP and IT, the effects on reproduction were also observed at PEC, and survival at 5PEC. In HR, effects on survival were observed at 5PEC. The toxicity observed in the FR and PT mixtures was higher than expected when considering the NOEC values of the single pesticides reported by EFSA. For instance, at PEC level, the reproduction was decreased in 90-95% of the control. The mixture composition of most toxic CSS (ES/IT, FR, PT) had the insecticide chlorantraniliprole, which is highly toxic to springtails, and the fungicides boscalid and difenoconazole. The results suggest a possible mixture effect due to the combination of chlorantraniliprole and azole fungicides. This work provides new information on the effects of realistic pesticide mixtures, which aims to contribute to a comprehensive assessment of their potential risks to soil organisms.

4.12.P-We393 Effects of Typical Pesticide Mixtures Representing European Agriculture on Soil Microbial Activity

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Microbial activities play an essential role in the decomposition and cycling of nutrients, and therefore are a good bioindicator of the health status of soils. The aim of this work was to assess the effects of 11 pesticide mixtures on C and N mineralization. Pesticide mixtures were selected based on their occurrence in soils from 11 case study sites (PT-Portugal, SP-Spain, FR-France, CH-Switzerland, IT-Italy, HR-Croatia, SI-Slovenia, CZ-Czech Republic, NL-The Netherlands, DK-Denmark, and AR-Argentina), together with their ecotoxicological data retrieved from EFSA reports. Soils were spiked with the pesticide mixture at the median measured environmental concentrations (MEC), predicted environmental concentration (PEC), and 5 times PEC (5PEC). After 28 days of incubation, C mineralization was measured using basal respiration (BR) and substrate-induced respiration (SIR), and N mineralization was assessed as the potential of ammonification (PAMO) and potential of ammonia oxidation (PAO). If the measured endpoints in the pesticide-treated soils indicated deviation higher than $\pm 25\%$ from controls, the measurements were repeated in 28-day intervals (i.e., 56 and 84 days). Deviation from solvent control $> \pm 25\%$ were observed in 6 out of the 11 mixtures after 28 days for at least one endpoint. For BR, the CZ mixture caused effects at PEC and 5PEC and PT mixture at 5PEC. In two treatments, PEC in PT and 5PEC in SP/IT mixtures, the differences were above the $\pm 25\%$ only when compared to the negative control. After 56 days, no effects were longer observed in any treatment, except in the PT mixture where deviation from control decreased to $< 25\%$ only after 84 days. SIR showed deviation $> 25\%$ at 5PEC in FR and CH mixtures after 28 days. After 56 days, the deviations from control were below $\pm 25\%$. PAMO decreased at PEC and 5PEC exposures in the CZ and NL mixtures. After 56 days, only in CZ mixture were the deviations $> 25\%$, however effects were no longer present after 84 days. PAO was only affected in the CZ mixture at PEC and 5PEC, and after 56 days, no effect was observed. Although the effects on C and N mineralization persisted up to 56 days in the most toxic mixtures (CZ for C mineralization and PT for N mineralization), the microbial activity recovered after 84 days.

4.12.P-We394 Toxicity and Bioaccumulation of Realistic Pesticide Mixture in Earthworm-Plant-Soil Microcosm

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In this work, a microcosm experiment was conducted to assess the bioaccumulation in earthworms and plants and toxicity on earthworms, plants and microbes for realistic pesticide mixture. Five pesticides were selected based on the frequency of occurrence in agricultural soils from the Czech Republic and their risk to soil invertebrates retrieved from EFSA documents. The mixture contained azoxystrobin, boscalid, dimoxystrobin, lambda-cyhalothrin, and thiophanate-methyl. Three concentrations of the pesticide mixture were prepared in 4 replicates: the medians of the measured concentrations (MEC) of the 5 selected pesticides, the predicted environmental concentrations (PEC) initially occurring in soil after pesticide application as retrieved from EFSA documents, and 5 times PEC values (5PEC). Ten adult earthworms with developed clitellum and 8 pre-germinated lettuce seeds were added to each microcosm pot, containing 1 kg of moist soil. After 14, 28, and 56 days, 4 pots of each treatment were sacrificed. The number of survival earthworm, and the biomass of earthworms and lettuce leaves and roots were measured. Depurated earthworms and lettuce roots and shoots were collected for analysis. The microbial activity was measured as carbon and nitrogen mineralization. At 56 days, the number of earthworm juveniles and cocoons were counted. Earthworm survival was only affected at 5PEC, where 90% and 100% mortality were observed after 28 and 56 days, respectively. Earthworm biomass and reproduction significantly decreased at PEC and 5 PEC. No effect on lettuce biomass was observed. The basal respiration was the most sensitive endpoint for microbial activity, with effects observed at MEC, while for the other endpoints (i.e., substrate induced respiration, potential ammonification, and potential ammonium oxidation) were affected at PEC and/or 5PEC. The uptake kinetics in earthworms was modeled using a first-order one-compartment model. It showed a potential for bioaccumulation in earthworms for boscalid and dimoxystrobin, with bioaccumulation factors above 1 in all concentrations. In lettuce root, beside boscalid and dimoxystrobin, azoxystrobin also showed a potential for bioconcentration. Lettuce leaves, on the other hand, showed much lower bioconcentration potential, indicating a limited translocation from roots to leaf. This work highlights the impact of pesticide mixture exposure, as deleterious effects were observed at realistic concentrations, such as MEC and PEC.

4.12.P-We395 Simulated Pesticide Spray Regime in Five Cropping Systems on Soil Invertebrates: Effects of a Cumulative Pesticide Mixture on European Native Earthworms

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To protect the environment, pesticide risk assessment is conducted, but it is often based on single pesticide

toxicity data. In agroecosystems, different pesticides are usually applied serially during growing seasons, leading to cumulative mixtures of pesticides in soil or other environmental matrices. Moreover, pesticides are registered based on the active substance, but formulations are applied in the field. It is, therefore, essential to know how pesticide formulations, following standard pesticide application patterns in the field, may cause mixture toxicity effects on soil biodiversity and ecosystem function. For our study, we exposed European native earthworm species (*Lumbricus rubellus* and *Lumbricus terrestris*) to pesticides based on pesticide spray schedule for five different cropping systems (CS). Glass jars of 500g, 50% water holding capacity (WHC) moistened soil in 5 replicates were prepared for each pesticide spray schedule for *L. rubellus*. For *L. terrestris*, an anecic and bigger-sized earthworm, glass framed mesocosms of 1.8 kg of 50% WHC moistened soil were prepared in 5 replicates. Dried horse manure mixed with oats were added as food for *L. rubellus* and litter made up of black poplar (*Populus nigra*) leaves were added as food on the soil surface for *L. terrestris*. To simulate a realistic field study, the recommended application rates of pesticide formulations were applied by spraying on the soil surface according to spray schedule on *L. rubellus* and *L. terrestris* for the duration ranging between 57 days to 98 days of the growing season typical to each CS. At the test end, relative to the control, the reproduction of *L. rubellus* was significantly impacted in all CS and the growth of *L. terrestris* was affected in all CS. Growth, mortality, and cast production of *L. terrestris* were consistently significantly impacted in a particular CS. The CS with the most pesticides applied carried more risk.

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4.12.P-We396 Health Risk Assessment in Agricultural Areas: Case Study of the Statte Area

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The characterization activity of some areas intended for agri-food production, located in the Territory of Statte (TA), conducted by the municipal administration of Statte in collaboration with the CNR of Bari, has highlighted exceedances of the legal limit reported in the Ministerial Decree 46/2019, for the organic micropollutant parameter (PCDD + dioxin-like PCBs) in soil samples. Therefore, in accordance with the provisions of this environmental law, the Health Risk Assessment (HRA) connected to the contamination of the site was carried out, starting from the development of the Preliminary Conceptual Model (PCM). This is aimed at identifying the possible sources of contamination, hypothesizing the mechanisms and processes of transport of the contaminants, and identifying the targets and the relative methods of exposure to potential contamination, allowing to evaluate the risk conditions for human health and the environment. To this end, by relating the results of the previous characterization analyses and those deriving from the implementation of the recent characterization of the soils collected in the survey area, with the geological and hydrological structure of the site and the land use of the areas investigated, we proceeded to analyze the elements that define the PCM and, therefore, to produce a preliminary health and environmental HRA. To assess the risk associated with the consumption of agri-food products from the agricultural areas of interest, specific monitoring criteria and sampling protocols were defined for plant biomass and agri-food products which, in consideration of the prevalent use of the land, involved vineyards and olive groves. Based on the analytical results of the agri-food products (grapes and olives), we then proceeded to assess the risk associated with their consumption, developing a risk assessment according to the methods set out in Annex 3 of the Ministerial Decree (46/2019). The health risk assessment, conducted for the grape and olive samples, highlighted that the risk deriving from the presence of the substances investigated is acceptable. These preliminary results are encouraging and do not highlight significant risks to human health following the intake of these agri-food products.

4.12.P-We397 Sustainable Integrated Management of Soil Nematode Control- The Responsibility of Choices

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Italy is characterized by a high marked value agro-industrial system, that relies on high value, quality and technological production, with increasing attention to sustainability. An agriculture that, however, still requires significant use of production factors as pesticides, which must be properly managed. This is particularly true in soil-borne parasitic nematodes control that, despite several proposed alternatives, still

rely on chemicals as soil fumigants, due to their impact and severe losses in high value crops grown under intensive growing system. Current legislation, which mandates sustainable agriculture, does not impose the choice of strategy to be employed, leaving farmers with the responsibility to decide within a wide range of possibilities. However, the sustainable use of fumigants, given the complexity of the subject, the great uncertainty on the effectiveness of alternative treatments to chemicals, due to the lack of eradicant effect and, for high value crops, the scale of investments required, necessitates a multi-stakeholder approach involving various parties beyond just farmers, including both private sector and public entities. The proposal of this work is the production of a document agreed upon by multiple parties, that outline needs and critical issues, summarizes key ideas, and suggests necessary actions that could serve as a valuable tool for decision-makers, encouraging them to consider and support all effective solutions and techniques to achieve the European objectives regarding soil fumigants risk reduction and exposure, through proper implementation of IPM. To this end, a multi-phase approach combining several levels of information (1-consultation, to gather data with qualitative interview, 2- engagement, for sharing data, expert perspective and knowledge, 3-feedback) is proposed, following also the Expert Knowledge Elicitation EFSA guidance. The goal is to acquire qualitative data on how issues related to limiting and/or better targeting fumigant use against major soil-borne pathogens, are addressed by users, to develop and suggest strategies and tools for better and more effective management and communication regarding environmental and human s risk. The initiative emphasizes sustainable integrated nematode management as a cornerstone for achieving this goal. Results will feed a shared position paper aimed at facilitating dialogue among stakeholders, ensuring that diverse perspectives are included in decision-making processes

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4.12.P-We398 Mind the Gap: Balancing the Need for Regulatory Safety and Sustainability of Crop Protection Products

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Crop Protection Products (CPP) play an important role in agriculture by protecting crops from pests and maintaining crop yield, hence supporting the United Nations Sustainable Development Goals (SDGs) in promoting global food security.

In the EU, pesticides are regulated under Regulation (EC) No. 1107/2009 (EC 2009) which sets approval criteria to ensure human health and environmental safety. Though this regulation addresses safety, which is a key sustainability criterion, it does not explicitly address other aspects of sustainability or include additional incentives for more sustainable elements to be adopted in the design of crop protection products. However, recommendations on the use of CPP are provided in the Sustainable Use Directive (Directive 2009/128/EC) but their implementation and enforcement are uneven across the EU.

As the EU advances initiatives like the Green Deal and the Farm to Fork Strategy, there is a growing emphasis on sustainable agriculture. However, without clear sustainability criteria for crop protection products, it remains challenging to guide the development of products that are both safe and sustainable. Manufacturers typically focus on regulatory compliance and the registrability of agrochemicals during development of a product. However, having well defined sustainability criteria, would promote the adoption and incorporation of other sustainable attributes. This could also be supported by clear business incentives for manufacturers to ensure products meet defined sustainable advantages to accelerate investment into these product types.

To address this, we propose criteria for consideration when (re)designing agrochemicals based on the principles of the EU Safe and Sustainable by Design (SSbD) and additional criteria specific to the use and function of plant protection products, for discussion. These criteria include efficacy, reduced exposure, green chemistry principles, agronomic justification, compatibility with integrated pest management (IPM) and sustainable farming systems e.g. soil health. Establishing such criteria can help guide agrochemical development towards a future that aligns with EU policies and the UN SDGs, fostering resilient and sustainable agricultural practices.

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4.12.P-We399 Effects of Realistic Pesticide Mixtures on Wheat (*Triticum Aestivum*) And Lettuce (*Lactuca Sativa*)

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As primary producers, terrestrial plants are the base of the food web, producing energy for other trophic levels. Moreover, terrestrial plants are also responsible for providing habitat and shelter to other organisms and, in the case of leguminous plants, for hosting nitrogen-fixing bacteria. This work aimed to assess the effects of realistic pesticide mixtures to terrestrial plants using the soil as the exposure route to mimic the toxicity of soil residues. For each mixture, 5 pesticides were selected based on their risk to terrestrial plants (values retrieved from EFSA reports) and their frequency of occurrence in agricultural soils from 10 European countries (Portugal, Spain, France, Switzerland, Italy, Croatia, Slovenia, Czech Republic, The Netherlands, and Denmark) and Argentina. Root growth inhibition test (ISO 11269-1) and seedling emergence and growth test (OECD 208) were conducted with wheat (*Triticum aestivum*) and lettuce plants (*Lactuca sativa*). Three concentrations were used: median measured environmental concentration (MEC), predicted environmental concentration (PEC), and 5 times PEC (5PEC). The data was analyzed by a Kruskal-Wallis test, followed by a Dunn's post hoc test to compare the concentrations to the control. In wheat, root growth was the most sensitive endpoint, and a significant decrease compared to control was observed in 10 out of the 11 mixtures. In lettuce, biomass of seedling was the most sensitive endpoint with significant effects in 9 mixtures. Considering both species, significant effects in root growth or seedling biomass were observed at the MEC level in 4 mixtures (France, Switzerland, Slovenia, and The Netherlands). Significant effects on seedling emergence were observed at PEC level in 7 mixtures (Spain, Italy, Czech Republic, The Netherlands, and Argentina). The effects were species-specific and endpoint-specific, which can be related to the different mixture composition and mode of action of the herbicides in the mixtures. For instance, the mixtures that affected seedling emergence had microtubule assembly inhibitors (e.g., propyzamide, pendimethalin), or synthetic auxins (e.g., Mecoprop P and 2,4-D). Effects occurring at MEC indicate that the presence of multiple pesticide residues in soil is an important route of exposure to be considered.

4.12.P-We400 Towards Improved Risk Management to Reduce Chemical Pressure on Biodiversity in Europe

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Risk management (RM) is a core element of the different European chemical regulations (REACH, CLP, pesticides and pharmaceuticals). RM describes a process of identifying and implementing measures to prevent or reduce an environmental risk. At the same time, chemical pollution has become one of the greatest threats to biodiversity. There is already a discussion on how to better integrate the protection of biodiversity into environmental risk assessments (ERAs). What has not been discussed so far is the role that RM can play in the goal of better protecting biodiversity.

One challenge in answering this question lies in the fact that firstly, different understandings and procedures of RM exist (e.g. in terms of scope, principles, strategies, instruments, etc.). Secondly, the different chemical regulation systems do not have the same RM structures and procedures and use different types of risk management measures (RMM). Based on scientific literature and the concept of risk governance, we first propose a definition and characterization of RM and a distinction between different types of RMM. This provides a first descriptive analytical lens for characterizing the current RM structures and procedures. To deepen the analytical perspective towards gaps and problems in preventing the loss of biodiversity and identification of possible improvements of the implemented RM systems, the Biodiversity Policy Integration approach is used to analyze the current RM systems regarding the integration of biodiversity in a conceptual and procedural way.

Based on this analytical framework, we will take an explorative problem-oriented approach by using qualitative empirical social research methods. These include a document analysis as well as expert interviews with various stakeholders related to the different RM systems under consideration. We strive to unravel the RM logics, the practices of how RMM are applied and to identify problems in procedural and structural terms to avoid biodiversity loss and reduce pollution. By doing so, we will identify current challenges and gaps more systematically and identify possible intervention points for improvement. The overall aim of this poster is to present our analysis on the RM systems in a holistic way in the first place. Based on this basic review, we illustrate steps for systemic improvement and outline specific novel measures.

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4.13 Advances in Risk Assessment for Plant Protection Products With a Non-conventional Mode-Of-Action

4.13.T-01 Problem Formulation for the Risk Assessment of Low-Concern Pesticides

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In response to the European Green Deal and the Sustainable Use Directive the European authorities have introduced measures to reduce the use of chemical pesticides, and to encourage the introduction in the market and use of non-chemical pesticides. Non-chemical pesticides include those based on microorganisms and non-living substances such as Low-Concern Active Substances (LCASs; i.e., substances which are expected to be of low concern). However, the regulatory framework of LCASs is based on the framework for conventional synthetic active substances and plant protection products. Harmonised guidance on how to make this framework more appropriate and efficient for LCAS is lacking in most cases.

In the EFSA-funded project entitled Develop a stepwise approach for a fit-for-purpose risk assessment, in particular for low-concern active substances and uses our consortium has developed a risk assessment scheme based on Problem Formulation (PF) and Pathways to Breach the Protection Goal (PBPg) model. For this scheme, generic PBPg were drawn up for each combination of a hazard and protection goal. This scheme can be applied to all types of LCAS due to its inherent flexibility and aims to harmonise the approach for the assessment of LCAS. The scheme was applied to case studies representing the main types of LCASs (botanicals, semiochemicals, microbial metabolites, inorganics, peptides and dsRNA). The proposed problem formulation method using PBPg can be used at any stage of the risk assessment. During the pre-submission stage it can be used to determine the overall approach for the risk assessment, including which type of data is needed (e.g., literature data, guideline testing, customised testing). At this stage, upon generating this data, the problem formulation step can be repeated to include the new information and in this way determine the most appropriate next step (whether it is needed to generate further data or not). At the evaluation stage, the approach can be applied to the assessment regardless of whether the approach was used at the pre-submission stage. Also, the approach helps to present the outcome of the risk assessment in a transparent way, thereby facilitating risk communication with risk managers and stakeholders.

4.13.T-02 RNAi as a Novel Mode of Action of Plant Protection Products: How to Assess Risks for Soil Invertebrates?

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In the context of the European Green Deal, an increase in the development and market authorisation of sustainable low-risk plant protection products is a key factor. A natural origin of the active ingredient is often proposed as a possible way forward in this context. The mechanism of RNA interference (RNAi) by double-stranded RNA (dsRNA) is a natural post-transcriptional gene silencing process which is conserved in eukaryotes. The first dsRNA-based plant protection product has been authorized in the U.S. in 2023. This product targets Colorado potato beetles, which is also a major pest species in Europe. Consequently, the first product with dsRNA as active ingredient is up for seeking marketing authorisation in the European Union. The present contribution addresses the assessment of potential effects of dsRNA on soil invertebrates, a standard non-target organism group that needs to be considered in the environmental risk assessment in the European Union. The conceptual approach that we developed and followed will be presented and discussed. The first steps of the conceptual approach have already been completed by selecting the target dsRNA as well as (assumed) negative and positive reference dsRNA. Due to high gene sequence homology, the collembolan species *Folsomia candida* was selected in the present case as the most suitable soil NTO, and oral uptake was identified as the most relevant exposure route. The first tests

following an adapted protocol of the standardized collembolan reproduction test have been completed with reference dsRNA and tests are ongoing with target dsRNA. While effects were observed on the reproduction of the collembolans, it is currently under investigation whether they relate indeed to sequence-specific RNAi-mediated processes, or rather reflect other pathways of toxicity.

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4.13.T-03 The Effects of a Microorganism-Based Plant Protection Product on a Simple Aquatic Ecosystem

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Plant protection products (PPPs) utilizing microorganisms as active ingredient are considered promising alternatives to conventional pesticides due to their natural occurrence in the environment and attributed species specificity. These strains often do not show direct toxicity effects to standard aquatic test organisms, but information on their fate in water or sediment and their effects on aquatic environments, including the microbial community, remains limited. Additionally, it was reported that microbial pesticides may increase turbidity, potentially reducing light availability and affecting primary production, with cascading effects to the aquatic community. This study aimed to assess the direct and indirect effects of a PPP containing *Bacillus amyloliquefaciens* strain FZB42 on aquatic organisms and their interactions in an indoor microcosm setting. We hypothesized that the pesticide would not have direct toxic effects on ecosystem structure and function, but expected increased turbidity, altered microbial community, and indirect effects on the feeding of *Gammarus pulex* at high pesticide concentrations. The experiment involved three different treatments (1x or 4x application of the max. recommended dose and 1x application of 10x max. recommended dose) and three different controls (negative, filtered and deactivated) over four weeks. Measured endpoints included respiration, macrophyte biomass, microbial decomposition, phytoplankton chlorophyll-a, zooplankton and microbial community composition, macroinvertebrate survival and feeding, and abiotic parameters (nutrient levels, EC, pH, oxygen, and temperature). The microcosms were highly productive, resulting in low nutrient concentrations, increasing pH and high dissolved oxygen levels. Contrary to our hypotheses there were no significant differences in microbial decomposition, macroinvertebrate feeding, or turbidity across treatments. Two key endpoints, zooplankton and microbial community composition, have not been evaluated yet. The findings from this microcosm experiment provide new insights into the effects of microorganism-based PPPs on aquatic ecosystems, which can be implemented to improve the risk assessment and determine the focus points compared to conventional, synthetic pesticides. Indirect effects on microbial community or productivity of aquatic ecosystems may prove to be more relevant than classic, direct toxicity endpoints.

4.13.T-04 Microbial Biopesticides are Inhibited by the In Vitro Diet Used in Larval Honey Bee Laboratory Bioassays

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Royal jelly is a substance secreted by the hypopharyngeal glands of worker honey bees and is the primary component of the in vitro honey bee larval diet according to the OECD 239 test guidance. Royal jelly is a complex and nutrient-rich substance yet has several bioactive properties that may create barriers for the testing of microbial-based plant protection products. While establishing the safety of these materials is as important as for conventional chemicals, there are important distinctions between them. For example, microbes must be evaluated for their pathogenic potential because some microbes are known to only affect the larval stage of bees (e.g., *Paenibacillus* larvae, American Foul Brood). For characterizing microbial inhibition from in vitro larval diet, five microbes were grown on agar plates and the in vitro larval bee diet was spotted on the plates. Inhibition tests revealed that the diet inhibited the growth of some of the bacteria and fungi, including the known bee pathogen *Paenibacillus* larvae. This work highlights the need for considering adaptations to current standardized approaches, which may include prework to confirm whether the test material is inhibited when integrated into the royal jelly-based in vitro larval diet.

4.13.P-Th338 An RT-qPCR-Based Protocol for the Detection and Quantification of dsRNA Pesticides

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Double stranded RNA (dsRNA) pesticides, triggering RNA interference (RNAi) of selected targets, have recently emerged as an effective and eco-friendly solution to the challenges posed by climate change and the urgent need for sustainable agriculture. To assess the environmental fate of dsRNA pesticides, a sensitive, precise and versatile method for its detection and quantification is needed. Here, we report on a RT-qPCR protocol developed to tackle obstacles related with dsRNA s detection and quantification in complex matrices as soil, where both degradation and adsorption may complicate the interpretation of results. At first, the protocol is optimized for an in vitro quantification of dsRNA, using treatments to reduce interference associated with non-functional nucleic acids, such as DNA or single stranded RNAs, that may be present either in the dsRNA product or in the environment. Next, a multi-step protocol was applied in soil samples to facilitate the dsRNA recovery, after its adsorption to soil particles. Furthermore, when chitosan-based formulation was tested, it was shown to not interfere with its RT-qPCR detection. The proposed method may easily be adapted to suit any dsRNA sequence and can be employed for ecological risk assessment and regulatory evaluations.

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4.13.P-Th339 Microbial Effects on Honeybees: Impact of the Use of Pollen in the Diet of Honeybee's Survival in a 10-day Laboratory Chronic Oral Toxicity Test Prolonged to 30 Days

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Among non-synthetic plant protection products, microbial biopesticides represent more sustainable methods for pest management. These biocontrol agents are usually considered as safe for non-target species, such as pollinators. To date, specific ecotoxicological protocols to test their effects are still not available, even if a prolonged observation time after exposure is sometimes required (e.g. 30 days, OPPTS). The standard diet (sucrose solution) proposed by the current guidelines doesn't permit to achieve bees survival for more than 18 days. The lifespan in honeybees is strongly influenced by diet, especially regarding the content in proteins. Therefore, the best diet to keep bees alive for 30 days was investigated. In particular a preliminary experiment was carried out to assess the benefits of two different sources of proteins: pollen and synthetic essential amino acids (EAAs).

Our results demonstrated that a diet enriched with pollen guarantees an increase in honeybees survival. The 20% of organisms fed with only sucrose solution (50% w/v) died after 18 days. The mortality of honeybees fed with EAAs was 23.3 % after 15 days (EAAs 1:500), 23.3 % after 22 days (EAAs 1:250) and 20% at 14 days (EAAs 1:50). The mortality of honeybees fed with pollen was 10 % after 30 days. Based on these preliminary results, the impact of microbial biopesticide on honeybees was investigated by comparing the effects observed in organisms fed with only sucrose solution and honeybees fed also with pollen ad libitum. The test without pollen addition allowed to determine an EC₅₀ after 20 days equal to 61.7 x 10⁸ CFU/mL while the test performed with pollen addition showed an EC₅₀ respectively equal to > 405 x 10⁸ at 20 days and equal to 366.5 x 10⁸ CFU/mL after 30 days.

In this context, our results showed also a clear improvement in honeybees' survival in laboratory conditions (OECD 245, OPPTS) when feeding with a diet enriched with pollen. Furthermore, a mitigation of the negative effects was recorded by comparing the EC₅₀ obtained from both exposure routes. In conclusion, the current methods for evaluating microbial pesticides are not optimized, especially when prolonging the observation after 20 days. The development of standardized tests that can be used as lines of evidence in assessing the likelihood of adverse effects of microbial pesticides on bees is of particular interest and should be investigated further.

4.13.P-Th340 Risk Assessment Innovation for Low-Risk Pesticides with *Caenorhabditis elegans* as Ecotoxicological Model

Xupeng Yu and Nico van den Brink, Wageningen University, Netherlands

Current pesticide risk assessment protocols in the EU are primarily designed for chemical pesticides, with limited standardized approaches for low-risk pesticides. This study evaluates the impacts of three low-risk pesticides-- double-stranded RNA (dsRNA) targeting the Proteasome subunit beta 5 (PSMB5) in Colorado potato beetle (CPB), pelargonic acid (PA), and *Bacillus amyloliquefaciens*-- on the activity and development of *Caenorhabditis elegans*, as measured by WMicrotracker ONE. Our results showed that

dsRNA exposure rather than producing species-specific effects acting upon target genes, triggers a general immune response at high concentrations (>5 µg/ml) causing delayed development and/or lower activity. PA exhibited the observable effect at field-use concentrations and showed an increased impact in the second generation, suggesting a potential for long-term influence. Moreover, *C. elegans* displayed lower activity level even at low exposure of *B. amyloliquefaciens* (0.04 µl/ml), implying possible interference with natural organisms. These findings underscore the necessity for customized assessment protocols for low-risk pesticides and highlight *C. elegans* as a suitable model organism for sustainable pest management risk assessment.

4.13.P Advances in Risk Assessment for Plant Protection Products With a Non-conventional Mode-Of-Action

4.13.P-Th338 An RT-qPCR-Based Protocol for the Detection and Quantification of dsRNA Pesticides

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Double stranded RNA (dsRNA) pesticides, triggering RNA interference (RNAi) of selected targets, have recently emerged as an effective and eco-friendly solution to the challenges posed by climate change and the urgent need for sustainable agriculture. To assess the environmental fate of dsRNA pesticides, a sensitive, precise and versatile method for its detection and quantification is needed. Here, we report on a RT-qPCR protocol developed to tackle obstacles related with dsRNA s detection and quantification in complex matrices as soil, where both degradation and adsorption may complicate the interpretation of results. At first, the protocol is optimized for an in vitro quantification of dsRNA, using treatments to reduce interference associated with non-functional nucleic acids, such as DNA or single stranded RNAs, that may be present either in the dsRNA product or in the environment. Next, a multi-step protocol was applied in soil samples to facilitate the dsRNA recovery, after its adsorption to soil particles. Furthermore, when chitosan-based formulation was tested, it was shown to not interfere with its RT-qPCR detection. The proposed method may easily be adapted to suit any dsRNA sequence and can be employed for ecological risk assessment and regulatory evaluations.

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4.13.P-Th339 Microbial Effects on Honeybees: Impact of the Use of Pollen in the Diet of Honeybee's Survival in a 10-day Laboratory Chronic Oral Toxicity Test Prolonged to 30 Days

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Among non-synthetic plant protection products, microbial biopesticides represent more sustainable methods for pest management. These biocontrol agents are usually considered as safe for non-target species, such as pollinators. To date, specific ecotoxicological protocols to test their effects are still not available, even if a prolonged observation time after exposure is sometimes required (e.g. 30 days, OPPTS). The standard diet (sucrose solution) proposed by the current guidelines doesn't permit to achieve bees survival for more than 18 days. The lifespan in honeybees is strongly influenced by diet, especially regarding the content in proteins. Therefore, the best diet to keep bees alive for 30 days was investigated. In particular a preliminary experiment was carried out to assess the benefits of two different sources of proteins: pollen and synthetic essential amino acids (EAAs).

Our results demonstrated that a diet enriched with pollen guarantees an increase in honeybees survival. The 20% of organisms fed with only sucrose solution (50% w/v) died after 18 days. The mortality of honeybees fed with EAAs was 23.3 % after 15 days (EAAs 1:500), 23.3 % after 22 days (EAAs 1:250) and 20% at 14 days (EAAs 1:50). The mortality of honeybees fed with pollen was 10 % after 30 days. Based on these preliminary results, the impact of microbial biopesticide on honeybees was investigated by comparing the effects observed in organisms fed with only sucrose solution and honeybees fed also with pollen ad libitum. The test without pollen addition allowed to determine an EC50 after 20 days equal to 61.7 x 108 CFU/mL while the test performed with pollen addition showed an EC50 respectively equal to > 405 x 108 at 20 days and equal to 366.5 x 108 CFU/mL after 30 days.

In this context, our results showed also a clear improvement in honeybees' survival in laboratory

conditions (OECD 245, OPPTS) when feeding with a diet enriched with pollen. Furthermore, a mitigation of the negative effects was recorded by comparing the EC50 obtained from both exposure routes. In conclusion, the current methods for evaluating microbial pesticides are not optimized, especially when prolonging the observation after 20 days. The development of standardized tests that can be used as lines of evidence in assessing the likelihood of adverse effects of microbial pesticides on bees is of particular interest and should be investigated further.

4.13.P-Th340 Risk Assessment Innovation for Low-Risk Pesticides with *Caenorhabditis Elegans* as Ecotoxicological Model

Xupeng Yu and Nico van den Brink, Wageningen University, Netherlands

Current pesticide risk assessment protocols in the EU are primarily designed for chemical pesticides, with limited standardized approaches for low-risk pesticides. This study evaluates the impacts of three low-risk pesticides -- double-stranded RNA (dsRNA) targeting the Proteasome subunit beta 5 (PSMB5) in Colorado potato beetle (CPB), pelargonic acid (PA), and *Bacillus amyloliquefaciens*-- on the activity and development of *Caenorhabditis elegans*, as measured by WMicrotracker ONE. Our results showed that dsRNA exposure rather than producing species-specific effects acting upon target genes, triggers a general immune response at high concentrations (>5 µg/ml) causing delayed development and/or lower activity. PA exhibited the observable effect at field-use concentrations and showed an increased impact in the second generation, suggesting a potential for long-term influence. Moreover, *C. elegans* displayed lower activity level even at low exposure of *B. amyloliquefaciens* (0.04 µl/ml), implying possible interference with natural organisms. These findings underscore the necessity for customized assessment protocols for low-risk pesticides and highlight *C. elegans* as a suitable model organism for sustainable pest management risk assessment.

4.13.P-Th341 Environmental Risk Assessment of RNAi-Based Plant Protection - A Literature Review

Udo Hommen¹, Kerstin Derz¹, Elke Eilebrecht¹, Kirsten Germing¹, Andreas Schiermeyer¹, Sebastian Eilebrecht¹ and Mathias Otto², (1)Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany, (2)BfN, Germany

Applications using the molecular mechanism of RNA interference (RNAi) are relatively new types of plant protection. The effect of RNAi is primarily based on a reduction in the expression of essential genes of the target organism, which is triggered by ingested double-stranded RNA (dsRNA) as the actual active ingredient. In such RNAi-based approaches, a distinction is made between endogenous and exogenous applications. Endogenous application means the production of the dsRNA in genetically modified plants (GMPs) and is also called host-induced gene silencing. In exogenous applications, dsRNA is used as an active ingredient in plant protection products. In most cases these are sprayed onto crops, which is also known as spray-induced gene silencing. The poster will present the results of one work package of a project commissioned by the German Federal Agency for Nature Conservation (BfN) on Environmental effects of RNAi-based GM plants and methods for transient modification of organisms. In the specific work package, we aim to determine the status quo of environmental risk assessment of RNAi-based GMPs and plant protection products and to identify existing gaps. The overarching complexes such as (1) the stability of dsRNA in the environment, (2) its uptake in non-target organisms, (3) its effects on non-target organisms as well as (4) the consequences of similarities and differences between endogenous and exogenous application for risk assessment will be addressed. Available environmental risk assessments of the US EPA for an RNAi based crop and an RNAi spray are summarized and compared to EU requirements for plant protection products.

4.13.P-Th342 Transcriptome Analysis of RNAi Spray Effects on the Aquatic Invertebrate *Daphnia magna* as a Non-Target Model Organism

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RNA interference (RNAi)-based pesticides represent a novel class of pesticides with a distinct chemical structure compared to conventional pesticides. Categorized as biopesticides, they are expected to have a reduced environmental impact and minimal effects on non-target organisms. RNAi is a post-transcriptional gene silencing process that naturally occurs in eukaryotic organisms, leading to sequence-specific suppression of gene expression in the target organisms. Gene silencing can affect growth, cause developmental defects, and can lead to mortality. The RNAi pathway suppresses gene expression in pests by inducing sequence-specific silencing through mRNA degradation via siRNA. This suggests that RNAi-based pesticides have little to no effect on non-target organisms and degrade rapidly in the environment due to the low stability of RNA molecules. However, this assumption is yet to be proven, as current risk

assessment may need adaptation to the molecular nature of RNAi pesticides.

The aim of this project is to assess whether a new designed RNAi-based pesticides affect the non-target organism *Daphnia magna*. In the first phase, acute tests with *D. magna* following OECD Guideline 202 are conducted. Juvenile *D. magna* are exposed to three different substances: 1) a RNAi-based spray formulation containing dsRNA targeting the p300 mRNA of aphids; 2) a RNAi-based spray formulation containing dsRNA targeting the Green Fluorescent Protein (GFP) and 3) the conventional pesticide flonicamid. The RNAi-based pesticide is the experimental substance on the field. The GFP formulation serves as a control. Any potential effects on *Daphnia* observed with the pesticide can be attributed to the spray formulation or dsRNA. Flonicamid, which has a similar mode of action to RNAi in aphids, will allow for a comparison of modes of action with the RNAi-based pesticide.

In the second phase, the acute tests will be repeated with a sublethal concentration range and the RNA of the exposed organism will be extracted. In order to identify the Mode of Action of the substances, a whole genome expression analysis of the RNA level will be conducted.

In the third phase, a method for concentration analysis of the dsRNA in the test medium via qPCR will be developed. In addition, the sequence homologies of the P300dsRNA in *D. magna* will be assessed via NCBI BLAST. This will help draw conclusions regarding the effects observed in the acute toxicity tests with the RNAi-based pesticide.

4.13.P-Th343 Impact of dsRNA-based Plant Protection Products on Aquatic Organisms

Judith Epping, Ruoying Song and Paul van den Brink, *Aquatic Ecology and Water Quality Management Group, Wageningen University, Netherlands*

Plant protection products (PPPs) with new, non-conventional modes-of-action provide a promising alternative to currently widely used synthetic pesticides. An emerging novel crop protection opportunity is the use of double-stranded ribonucleic acid (dsRNA). PPPs with dsRNA as active substance utilize the RNA interference mechanism by targeting specific genes to disrupt crucial physiological functions in the development, metabolism or reproduction of the pest or pathogen. Bioinformatic screening allows to design dsRNA sequences and consequently PPPs that are highly species specific. Additionally, dsRNA has a short degradation time in environmental matrices, which could minimize its impact on non-target organisms compared to conventional synthetic pesticides. In practice, little data is available about the effects of dsRNA on aquatic organisms. The objective of this research is to determine whether dsRNA affects standard aquatic test species and to which extent current test guidelines need to be adapted to assess their sensitivity to this PPP with non-conventional mode-of-action. Three different dsRNA sequences will be tested, one targeted at a common pest (Colorado Potato Beetle, CPB, PSMB5 gene), one negative control (green fluorescent protein, GFP) and one positive control sequence with a highly conserved gene (actin). All dsRNA will be tested in four concentrations ranging from 0.025 mg/L to 25 mg/L on four different test species, including *Daphnia magna*, *Chironomus riparius*, *Hyalella azteca* and an aquatic beetle (to be determined). Testing will closely follow OECD test guidelines 202 (*Daphnia*), 235 (*Chironomus*) and 321 (*Hyalella*) with an extended observation period of 96h. Mortality and immobility will be recorded at 24, 48 and 96h. RT-qPCR will be used to monitor dsRNA concentrations over time. We hypothesize that the PSMB5 and GFP gene sequences will not affect the different test organisms due to their species specificity and non-essential nature, respectively. As a positive control, we expect the dsRNA sequence targeting actin to also affect aquatic organisms. The results of this study will provide insights into the impact of dsRNA-based PPPs to aquatic organisms. The findings will also support the advancement of a risk assessment adjusted to the requirements of the novel, non-conventional mode-of-action of this group of PPPs.

4.13.P-Th344 Environmental Risk Assessment of RNA Interference Based Pesticides

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Currently novel pesticides are being developed on the basis of the biological process of RNA interference. Their mechanism of action differs significantly from that of chemically synthesized and other biological pesticides. A double-stranded RNA (dsRNA) is used, which is designed to be homologous to a sequence segment of the messenger RNA (mRNA) of a protein that is vital for the target organism. Through processing in the organism and the molecular biological process of RNA interference (RNAi), the mRNA is either degraded or its translation into the encoded protein is inhibited, resulting in the death of the target organism. To date, one RNAi-based pesticide has already been approved by the USEPA.

Due to the sequence specificity of the mechanism, RNAi pesticides are supposed to be highly specific and thereby should have no adverse effects on non-target organisms. Thus, they could contribute to a

sustainable plant protection. However, there are still many open questions and data gaps regarding the potential environmental risk of such substances. Whether adverse effects actually occur in non-target organisms, is not yet sufficiently understood and depends, among other things, on exposure, bioavailability and actual specificity. Due to the properties and modes of action of RNAi pesticides, both the data requirements and the risk assessment criteria for the EU authorization of chemically synthesized or biological pesticides are not appropriate and applicable to them and need to be adapted and/or newly developed. This project is intended to contribute closing the knowledge gaps and developing accepted data requirements and guidelines for authorization.

To address these challenges the first phase of the project intends to identify how different formulations of the dsRNAs can change the bioavailability for different organisms. We aim to develop a standard protocol for a positive and negative control to test the bioavailability of differently formulated dsRNAs for standard non-target organisms. Transcriptome analysis will be applied to assess potential off-target effects of the formulated dsRNA in the non-target organism. The project will contribute to evaluating whether the standard organisms for the risk assessment at hand are sufficient to evaluate RNAi pesticides.

The results of this project will contribute to the definition and development of adapted data requirements and guidelines for EU authorization of RNAi pesticides.

4.13.P-Th345 Assessing the Toxicity of Orange Assential Oil (d-limonene) on Soil-Dwelling Springtails (*Folsomia candida*)

Anna Huang, Bastian H. Polst, Steven Droge, Arrienne Master and Gertie Arts, Wageningen Environmental Research, Netherlands

Plant extract products, such as essential oils, are increasingly used as plant protection products offering an alternative to conventional synthetic pesticides. Among these, orange oil, primarily composed of d-limonene, is one of the most produced essential oils globally. When sprayed on crops for their protection, orange oil is likely to also reach the soil, exposing soil organisms to d-limonene through ingestion of contaminated food and contact with pore water and soil particles containing residues post-application. Lethal effects of the formulated product have been reported by EFSA for non-target (above ground) arthropods (NTAs), within a factor of two above the application rate, but showed no risk to earthworms. However, the potential effects of orange oil products on other soil organisms remain largely unexplored. In our preliminary study, we investigated the acute toxicity of orange oil to the soil-dwelling springtail *Folsomia candida*. The experiment was conducted in closed jars containing 30 g of Lufa soil. We applied the formulated product (60 g/L, approximately 95% d-limonene) to the soil and added 20 adult individuals per replicate across three doses: control, 25 mg/kg soil, and 250 mg/kg soil, with four replicates for each treatment. The jars were sealed after setup, and the experiment lasted 7 days. Results showed less than 10% mortality in the control group, approximately 50% mortality in the 25 mg/kg treatment, and 100% mortality in the 250 mg/kg treatment. Our preliminary results indicate that orange oil is toxic to soil organisms, with an LC50 value of 25mg/kg soil; additionally, the EFSA dossier reports a predicted soil concentration of 4.18 mg/kg.

Our results highlight the need for comprehensive future studies to fully assess the environmental impact of orange oil, including chronic endpoints related to reproduction. In addition, studies should examine effects on other NTAs and plants using a holistic approach that accounts for the chemical's properties, such as high volatility. For example, researchers could spray orange oil on soil and focus on deposition on non-target plant parts like stems and leaves, as well as its cumulative effects on soil organisms and non-target terrestrial species. Moreover, the difficult chemical properties of d-limonene, such as low water solubility, high sorption, and high volatility, must be considered, as they complicate chemical analysis and exposure quantification.

4.14 Pharmaceuticals in the Environment Innovations in Risk Assessment, Regulation, and the Science Globally

4.14.T-01 Assessing the Environmental Sustainability of Human Medicinal Products: A Proposed Medicinal Product Sustainability Index (MPSI)

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There are increased global concerns about the continued presence of pharmaceuticals in the environment and the risk that these residues of active pharmaceutical ingredients (APIs) pose to wildlife and human health. Whilst the environmental hazards and risks of human medicinal products (HMPs) are assessed as part of their marketing application they are not a barrier to market approval for HMPs. The environmental exposure, and their wider environmental impacts, are not limited to the patient use of these medicinal products. Pollution can occur at the point of bulk drug manufacture and formulation resulting in hot spots of API exposure where insufficient industrial wastewater treatment and dilution is in place. Improper

disposal of unused or expired medicinal products can also be a source of APIs into the environment. However, if we think about environmental sustainability in its widest sense API production is resource inefficient ($\ll 1\%$); most small molecule APIs have a process mass intensity (PMI; Kg of raw material used to make a Kg of API) of between 100-1000. For large molecule APIs like monoclonal antibodies (mAb) the PMI is on average 7700 Kg raw material per Kg mAb. Once registered the manufacturing process is rarely updated to improve its environmental sustainability over time. Healthcare contributes to c.5% of global greenhouse gas emissions (GHG), making it one of the largest sectors contributing to global warming. Healthcare providers are starting to explore frameworks for green procurement of HMPs. Many of these procurement initiatives are focused on comparing GHG footprints of HMPs to help reduce their Scope 3 GHG footprint. This presentation proposes a wider sustainable procurement approach and advocates for a medicinal product sustainability index (MPSI) that captures (i) GHG emissions along the entire value chain, (ii) water intensity along the entire value chain, (iii) water scarcity risks in the supply chain, (iv) water quality and pollution risks at the point of manufacture and formulation, (v) natural resource efficiency at a process and dose level, (vi) environmental hazards and risks of the API, (vii) the use of substances of very high concern (SVHCs) in the manufacturing process and excipients, and (viii) extended producer responsibility commitments. The MPSI will be based on simple metrics that allows environmental sustainability performance data to be compared within and across pharmaceutical classes.

4.14.T-02 Using High Resolution Mass Spectrometry Non-Target Screening Data to Detect Exposure Patterns of Pharmaceuticals in German Rivers

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The increasing presence of pharmaceuticals in freshwaters poses risks to aquatic ecosystems health and challenges the regulatory frameworks. This study investigates the utility of high-resolution mass spectrometry (HRMS) non-target screening (NTS) portals in the detection of pharmaceutical mixtures and supports pharmaceutical legislation and Early Warning Systems. In line with EU strategies such as the Chemicals Strategy for Sustainability, we integrate HRMS NTS data into monitoring programmes using the German NTS portal. The German NTS portal, developed by the German Environment Agency (UBA) and the Federal Institute of Hydrology (BfG), contains HRMS NTS data of surface water and suspended particulate matter (SPM) samples from the German Environmental Specimen Bank collected over two decades and enables retrospective and trend analyses.

The NTS portal uses spectral libraries for secure substance annotation, which improves usability by the authorities. It utilises a spectral library of over 1600 substances, of which around 30 % are pharmaceuticals. We analyse spatial and temporal trends of pharmaceuticals, highlighting ubiquitous exposure patterns such as for metoprolol and seasonal trends such as for clarithromycin. The combination of quantified HRMS-NTS data with predicted no-effect concentrations (PNEC) enables the calculation of risk quotients and the identification of toxic drivers that can be used in prioritization.

We compare the risk quotients of pharmaceuticals with other substance groups such as persistent organic pollutants (POPs) and provide insights into the environmental exposure of pharmaceuticals on the EU Water Framework Directive Watch List.

Strong correlations between market volumes of pharmaceuticals and concentrations in the environment highlight the need of including production data in prioritization efforts. Reliable PNECs derived from the authorisation procedure improve the interpretation of water monitoring data and risk assessment. By making the data FAIR and accessible to authorities, the NTS portal contributes to the understanding of pharmaceutical mixtures in aquatic environments and thus to effective chemical regulation and environmental protection.

4.14.T-03 Fate of Pharmaceuticals in Aquatic-riparian Food Webs at Sites Receiving Municipal Wastewater Discharges

John Fast and Karen A. Kidd, McMaster University, Canada

Many classes of pharmaceuticals are regularly detected in surface waters downstream of wastewater treatment plants (WWTPs) and they may be bioaccumulated by larval aquatic insects and transferred to riparian predators through insect emergence from aquatic ecosystems. This study quantified the concentrations of active pharmaceutical ingredients (APIs) and select metabolites in aquatic insects and a family of riparian spiders that relies heavily on aquatic insect prey to assess the fate of APIs in downstream ecosystems impacted by WWTP effluents. Aquatic larval and terrestrial adult insects representing different functional feeding groups and nearshore spiders were collected three times in the summer of 2022 upstream and downstream of four WWTPs along the Grand River, southern Ontario, Canada. A modified QuEChERS method with matching internal standards was developed and is being

used to compensate for interferences across multiple taxa and life stages. Sixteen pharmaceutical targets and select metabolites, which may also pose a risk to aquatic life, were selected for analysis and include antidepressants, anticonvulsants, antibiotics, and anti-inflammatories commonly detected in surface waters. Six APIs (Carbamazepine, Fluoxetine, Ibuprofen, Venlafaxine, Triclosan, Diclofenac) and 6PPD-q were consistently (>50% detection rate) found in larval insects from the family Hydropsychidae at all sites, and levels were higher in insects collected downstream than upstream of the points of discharge. Selected major metabolites for parent compounds were not detected above method detection limits within most sites or taxa. Larval insects total body burden (Σ APIs) ranged 180 230 ng/g ww across all sites. Adult insects collected during emergence events, after metamorphosis has occurred, had lower body burdens of APIs (15 28 ng/g ww) than their larval forms but also higher concentrations and a greater diversity of contaminants in samples from downstream sites. Spiders had similar profiles of APIs as larval insects, but Σ APIs (54 103 ng/g ww) were lower in spiders than aquatic larvae suggesting potential differences within riparian spiders aquatic diet or an increased rate of depuration. Overall, results support the movement of APIs from aquatic to terrestrial ecosystems via emergent insects, and highlight the importance of more complete food web studies to better understand the export of aquatic APIs to terrestrial predators with a varied diet of aquatic prey.

4.14.T-04 Assessing the Suitability of Recent EU Environmental Quality Standards that Account for the Ecotoxicity and Prevalence of Transformation Products

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Environmental Quality Standards (EQSs) are key regulatory tools in Europe for assessing potential chemical risks and controlling discharges to the water environment. The European Commission's (EC) recent EQS Proposal includes EQS for two pharmaceutical substances, carbamazepine and clarithromycin. The derivations for both substances account for transformation products (TPs), which is relatively unusual for EQS derivations in Europe. The derived EQS for clarithromycin has been modified based on available ecotoxicity data for its TPs, whilst for carbamazepine the EQS is for the sum of the parent substance and metabolites measured in exposure data.

Here, we review the EC's EQS derivations for carbamazepine and clarithromycin to assess the suitability of the approaches for accounting for the TPs, comparing these approaches to the guidance and the available scientific evidence. For each substance, we identified any additional relevant and reliable ecotoxicity data on the parent compound and its TPs. We then derived deterministic freshwater EQS values and investigated where probabilistic approaches could be applied, including how to incorporate TP data into this approach.

Based on our review of the EC's EQS dossiers and our own derivations, we developed a roadmap for the incorporation of TP data into the derivation of EQSs for contaminants of emerging concern. The roadmap indicates how TP data could be considered based on its availability, relevance, reliability, and ecotoxicity and prevalence compared to the parent compound.

Based on an evidence-driven review of the EQS derivations for clarithromycin and carbamazepine and thorough assessment of the consideration of TP data, we reached the following conclusions: Some, but not all, contaminants of emerging concern have relevant and reliable data on their TPs which may need to be considered for EQS derivation.

Ecotoxicity and monitoring data on TPs are often scarce. Obtaining additional data on TPs would allow a more comprehensive evaluation of their hazards and exposure potential.

As the need to consider TPs is likely to become more common in regulatory assessments, it highlights the need to implement a coherent and consistent approach for how to incorporate TP data into such assessments and the derivation of evidence-driven and environmentally relevant limit values.

4.14.T-05 A Tiered Secondary Poisoning Risk Assessment Approach to Prevent Unnecessary Fish Bioaccumulation Testing of Pharmaceuticals

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Secondary poisoning risk assessment is a new requirement under the revised EMA guideline on environmental risk assessment of pharmaceuticals. The guideline triggers a fish bioaccumulation study when the logD_{ow} of the substance exceeds 3 at an environmentally relevant pH (5 to 9). When the resulting bioconcentration factor (BCF) is ≥ 100 L/kg, a secondary poisoning risk assessment is needed. Inputs for the calculation of secondary poisoning potential are fish BCF, chronic mammalian toxicity data, and the estimated concentration of API in surface waters. Extensive mammalian toxicity data and reliably conservative surface water exposure estimates are already available by the time a secondary poisoning risk

assessment is triggered. Therefore, we propose a modification to the secondary poisoning risk assessment approach prescribed by EMA, whereby the relevant mammalian toxicity data and guideline-compliant conservative exposure estimates are leveraged in order to determine the need for a fish bioaccumulation study, and to allow a conclusion on potential secondary poisoning risk.

In a first step, a worst-case secondary poisoning risk quotient is calculated using the equations described in the EMA guideline, but replacing the experimental BCF value with a conservative default BCF value based on existing, recently published datasets (Constantine et al., 2024; Gimeno et al., 2024;). These proposed default BCF correspond to the highest/percentile P95th values of available pharmaceuticals and other chemicals with logDow values comparable to that of the substance to be assessed. If the outcome of this preliminary step is a risk quotient below 1, conducting a fish bioaccumulation study would not be justified. On the other hand, a preliminary risk quotient (RQ) >1 derived in this manner would show the need for a substance-specific fish BCF to inform the secondary poisoning risk assessment.

For compounds yielding preliminary RQs around 1, additional sources of information, such as in vitro mammalian clearance data obtained during drug development, in vitro fish clearance data (e.g., OECD TG 319), or evidence for dose accumulation in preclinical studies, could further inform on the likelihood of a risk of secondary poisoning.

Several real examples are provided to illustrate this concept, and which, most importantly, highlight how a considerable amount of unnecessary fish testing could be avoided.

4.14.P Pharmaceuticals in the Environment Innovations in Risk Assessment, Regulation, and the Science Globally

4.14.P-Tu363 Integrating Predictive Modelling and Wastewater-Based Epidemiology for Comprehensive Environmental Risk Assessment of Pharmaceutical

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This study presents an innovative approach to environmental risk assessment (ERA) of pharmaceuticals by combining wastewater-based epidemiology and predictive modelling. The research addresses key challenges in API risk assessment, including the lack of quality hazard and exposure data, and the need for improved methodologies in predicting environmental concentrations.

The study utilized prescription data from England's National Health Services to predict pharmaceutical concentrations in wastewater influent/effluent and river. The study focused on pharmaceuticals across different therapeutic classes. Initial concentrations (PEC) of pharmaceuticals, which were then validated against measured environmental concentrations (MEC) from two wastewater treatment plants in Southwest England.

Key findings include: Accuracy of PEC predictions was assessed using the criterion ($0.5 < \text{PEC}/\text{MEC} < 2$) which reflects a close alignment between PEC and MECs: For influent, accurate predictions for 12 of 33 pharmaceuticals at site A and 15 of 33 at site B. For effluent, it is 11 of 28 pharmaceuticals at site A and 13 of 28 at site B. For river, it is 11 of 26 pharmaceuticals across both sites.

When PEC and MEC were used to rank the detected pharmaceuticals based on risk, generally both the approaches provided similar ranking, and this provides some confidence in the use of PEC in risk assessment of the pharmaceuticals.

The study also conducted a comprehensive ERA by calculating RQs using both predicted and measured concentrations, as well as PNEC-ENV and PNEC-MIC values. This approach allowed for a more holistic assessment of environmental risks across different trophic levels.

Results showed varying levels of environmental risk across different pharmaceuticals and trophic levels. Some compounds, like metronidazole, presented low risk, while others, such as clarithromycin and trimethoprim, consistently showed high risk. The study highlighted the importance of considering both PNEC-ENV and PNEC-MIC values for a comprehensive ERA approach.

The study underscores the need for refined predictive tools and standardized ERA procedures to address the environmental impact of pharmaceuticals in aquatic ecosystems. It also emphasizes the importance of monitoring data to improve the accuracy of risk assessments for specific regions, aligning with the session's focus on integrating the best available science and strategic approaches to managing APIs in the environment.

4.14.P-Tu364 Suspect Screening of Pharmaceutically Active Compounds in River Ecosystem by High-Resolution Mass Spectrometry

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Most of freshwater ecosystems in developed countries receive pharmaceutically active compounds (PhACs) from wastewater treatment plants (WWTPs), exposing them to the toxic effects these pollutants can cause. In this study, an analytical methodology based on Ultra High-Performance Liquid Chromatography coupled to High-Resolution Mass Spectrometry (UHPLC-QTOF HRMS) was developed to perform a suspect screening analysis to identify PhACs present in the river ecosystem. In this way, a more complete picture of the current PhAC contamination situation can be obtained, overcoming the limitations of target analysis methods.

173 samples were collected during 2020, 2021 and 2022 along the Tagus River basin (Spain) ecosystem: 89 surface river waters, 28 river sediments, 24 fish and 32 samples from WWTPs. WWTP samples comprised both wastewater and sewage sludge to qualitatively assess potential PhACs removal and water-sludge partition occurring during the treatment.

MS and MS/MS data following the chromatographic separation were registered in ESI positive ionization mode using data-independent acquisition. Identification criteria were based on more than a 75% match with libraries, a maximum accurate mass error of 5 ppm, and an isotopic pattern difference below 20%. Furthermore, only features with an intensity ten times higher than the blank intensity were considered. More than 100 PhACs were identified across all samples with a level of confidence of 2a according to Schymanski's scale.

Among all identified PhACs, the main therapeutic groups were psychiatric drugs (e.g., amisulpride), anti-inflammatories (e.g., diclofenac), analgesics (e.g., tramadol), and cardiovascular drugs (e.g., telmisartan). Surface water samples presented more than 20 PhACs identified in over 50% of the samples. Up to 54 compounds were identified in river sediments with detection frequencies between 4% and 61%. Fish was the matrix with the lowest number of PhACs identified and detection frequencies <35%. At least 60 PhACs were identified in wastewater samples revealing the transfer of some of these contaminants since more than 50 compounds were found in sewage sludges with detection frequencies up to 80%.

Transformation products resulting from drug metabolism were also explored, and metabolites of the antiepileptic carbamazepine and the hypnotic zolpidem were the most frequently detected (>55%).

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4.14.P-Tu365 Spatiotemporal Dynamics and Ecological Risks of Pharmaceuticals and Personal Care Products in Korean Rivers

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Pharmaceuticals and personal care products (PPCPs) are emerging contaminants of concern due to their pseudo-persistence in aquatic environments, resulting from continuous release and incomplete removal during wastewater treatment. This study investigated the occurrence, seasonal dynamics, and ecological risks of 137 PPCPs in four major Korean rivers Han, Geum, Yeongsan, and Nakdong over a one-year period from April 2020 to March 2021.

Water samples were collected monthly from 22 sites across the four rivers. PPCPs were analyzed using liquid chromatography-high resolution mass spectrometry (LC-HRMS). The ecological risks of PPCP mixtures were assessed by calculating the Risk Quotients based on the Sum of Toxic Units (RQSTU) for fish, crustaceans, and algae.

Of the 137 targeted PPCPs, 120 were detected, with 98 quantified above the limit of quantification. Concentrations varied significantly across seasons and rivers, ranging from a few ng/L to 42.7 µg/L for metformin. The highest mean summed concentration was observed in the Yeongsan River (22.5 µg/L), followed by Han (12.4 µg/L), Nakdong (11.9 µg/L), and Geum (10.9 µg/L) rivers. However, when considering total load based on monthly flow rates, the Han River contributed the highest pharmaceutical flux (607.7 kg/day), significantly exceeding other rivers.

Seasonal analysis revealed the highest concentrations in winter (20.5 µg/L), followed by spring (18.5 µg/L), summer (10.7 µg/L), and fall (10.1 µg/L). This pattern suggests reduced dilution during base flow conditions in winter and spring, contrasting with increased dispersion during summer and fall flood events.

Median seasonal RQSTU values were 5.4, 4.5, and 3.5 for fish, crustaceans, and algae, respectively, indicating moderate to high ecological risks. Seasonal analysis revealed the highest risks in spring, followed by fall, winter, and summer. This pattern suggests that certain high-risk PPCPs are less influenced by seasonal variations or flow conditions than overall concentrations.

This study highlights the importance of integrating spatiotemporal dynamics in PPCP risk assessments. The findings provide valuable insights for developing targeted monitoring and management strategies to mitigate ecological impacts in freshwater ecosystems, especially during high-risk seasons.

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4.14.P-Tu366 Pharmaceutical Contamination in European Groundwater and Drinking Water

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Pharmaceuticals and their transformation products are especially relevant Contaminants of Emerging Concern (CECs) due to their engineered bioactivity and their widespread presence in diverse environments. Pollution from pharmaceutical compounds has been detected in drinking water, and its precursor in Denmark and many other locations; groundwater. Understanding the chemical exposome related to consistent low-level contamination by pharmaceuticals in aquatic matrices is crucial due the potential toxicological implications and the ubiquitous nature of this exposure, even for vulnerable groups. With this goal in mind, this study first aims to summarize the extent of pharmaceutical pollution in European drinking and groundwater by compiling data from studies that have detected such compounds via two principle analytical methods, Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS). This study reports concentration ranges and detection frequency and dives into analysis of key pharmaceuticals, including carbamazepine, ibuprofen, diclofenac, sulfamethoxazole, metformin and phenazone. To further expand understanding of the chemical exposome, the study will analyse drinking water samples from Denmark using non-target analysis. LC-MS/MS will be utilized due to its high sensitivity and ability to distinguish between co-eluting compounds with the same molecular masses but different product ions. This technique will be used with spectral libraries to enable the probable identification of a wide array of compounds, providing broad insight into pharmaceutical contaminants. This approach aims to minimize research bias that could lead to the frequent detection of commonly identified compounds, thereby offering a more comprehensive exposure assessment and identifying additional chemicals of interest. Additionally, a toxicological perspective will be included as QSAR modelling and machine learning will be utilized to make relevant toxicological predictions, contributing to preliminary risk assessment. Overall, this study will contribute to the current understanding of the pollution load of pharmaceuticals in Danish drinking water and groundwater, giving a better picture of the human exposure to these compounds and their implications for human health. This research can serve to inform decisions regarding pharmaceutical regulation, pollution management, or water treatment optimization, to ensure the safety of this vital resource.

4.14.P-Tu367 Evaluation of Environmental Risks Posed by Pharmaceutically Active Compounds in the Great Bačka Canal

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The assessment of risks linked to the occurrence of pharmaceutically active compounds (PhACs) in aquatic environments has become a significant concern due to their potential impact on ecosystems and human health. The extent of PhACs presence in the Great Bačka Canal, known as one of the most polluted waterways in Europe, is not known, even though it is impacted by the unregulated discharge of various contaminants of emerging concern either from agricultural runoffs, wastewater effluents, or various industrial activities. This study aims to assess the risks associated with the presence of PhACs in the Great Bačka Canal through an evaluation of their concentration levels, and potential ecotoxicological effects. Forty surface water samples were collected along the Great Bačka Canal and analysed for a range of commonly used pharmaceuticals, including antibiotics, anti-inflammatory drugs, antidepressants, beta blockers, anti-epileptics, and lipid regulators. The ecological risk evaluation of detected PhACs was estimated based on the technical guidance document on risk assessment released by the European Commission. According to the mentioned document, the RQ risk model was assessed by the maximum environmental concentration (MEC) of individual pharmaceuticals measured in the surface water of the Great Bačka Canal divided by the predicted no-effect concentrations (PNEC) of pharmaceuticals. The risk posed to aquatic organisms was categorized into three levels: $RQ < 0.1$, low risk; $0.1 \leq RQ < 1$, moderate risk; $RQ \geq 1$, high risk. The findings indicate varying levels of pharmaceutical occurrence, with some compounds exceeding threshold concentrations known to cause ecological risk. The results underscore the need for improved wastewater treatment practices and the establishment of more stringent regulations to mitigate the impact of PhACs on the aquatic environment of the Great Bačka Canal. Furthermore, this research enhances the understanding of the environmental risks posed by pharmaceutical pollutants in freshwater systems, especially those used for crop irrigation.

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4.14.P-Tu368 Assessing the Environmental Risk of Pharmaceuticals in the Italian Waters: An Integrated Approach

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Environmental risk assessment (ERA) of pharmaceuticals is an essential process aimed at safeguarding our ecosystems from potential harm caused by medicines. The present project aimed to assess the environmental risk of pharmaceuticals in Italian waters by using two complementary approaches.

On one hand we assessed the environmental potential risk by collecting physico-chemical characteristics and (eco)toxicological data for more than project 300 compounds used in pharmaceutical products (such as APIs, preservatives, artificial sweeteners, dyes as well as some selected stable metabolites). The list of substances has been created by a prioritization process that involves the collection of actually measured drugs in Italian waters, the official consumption data and expert judgments of medical specialists from various therapeutic sectors. The data have been collected from different databases and they have been organized in technical sheets. A scoring system has been developed in order to calculate a presumed level of potential environmental risk for each substance, which is intrinsic to the molecular structure and independent from the geographical consumption or diffusion.

On the other hand, we planned to measure the actual concentrations of the listed substances in the Italian surface, ground and drinking waters, by sampling more than 100 site along the Italian peninsula aiming to cover the variety of water body geographical typologies, pollution pressures and water uses. The availability of measured concentrations will allow to calculate the site-specific risk using the risk quotient approach as the ration between measured concentrations (MEC) and PNEC of specific end-points.

The integration of database of concise fact sheets and site specific risk evaluation is the basis to develop a web-based application which can be freely consulted by health professionals in order to raise awareness on the environmental risks of pharmaceuticals preparations for doctors and pharmacists involved in drug prescriptions or recommendations.

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4.14.P-Tu369 Pharmaceutical Removal Via Anaerobic Digestion in Different Wastewater Matrices: Kinetic Assessment, Ecotoxicological Effects and Biotransformation Products

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While municipal and hospital wastewaters (MW and HW) share similar input sources, primarily from sanitary effluents, their compositions differ significantly, especially in terms of organic matter, nutrients, and contaminants of emerging concern. Many studies use synthetic wastewater (SW), often rich in standard carbohydrates and proteins, to control experimental conditions. However, comparisons between synthetic and real wastewater matrices are scarce. This study assessed anaerobic digestion performance for eight pharmaceuticals (acetaminophen-ACT, atenolol-ATN, caffeine-CAF, carbamazepine-CBZ, diclofenac-DCF, ibuprofen-IBU, naproxen-NPX, and propranolol-PRP), biogas production, ecotoxicological effects and biotransformation products across MW, HW, and SW matrices. Batch bioreactors were used with uniform agitation, temperature, and food-to-microorganism ratio. Biogas evolution, chemical oxygen demand (COD), and target compound levels were periodically monitored. Pharmaceuticals products were analyzed using tandem mass spectrometry and biotransformation products (TPs) via high-resolution mass spectrometry. Acute ecotoxicity tests were conducted on *C. silvestrii* and *D. similis* under varying dilutions (100, 50, 25%) before and after treatment. Methane production followed

a sigmoidal pattern for SW and MW, while HW showed a linear trend. Maximum methane production and production rate were highest for SW, followed by MW and HW. Variations in substrate concentrations likely influenced these outcomes, with HW exhibiting significantly lower COD levels compared to MW and SW. The presence of diverse pharmaceuticals, especially antibiotics in HW, may further inhibit methane production. Macro-nutrient (carbohydrates and proteins) removal efficiencies were higher for SW compared to MW and HW. Differences in initial macro-nutrient levels likely contributed to these trends, with SW exhibiting significantly higher carbohydrate and protein concentrations. CAF and NPX exhibited zero-order kinetic decay for CAF in SW, MW and HW. ACT followed a first-order decay pattern. Other compounds (ATN, CBZ, DCF, IBU, and PRP) showed no removal. TPs are under analysis. Ecotoxicity tests revealed *C. silvestrii* to be more sensitive than *D. similis*, with higher toxicity observed in SW compared to real matrices. Despite providing controlled conditions, the simplicity and higher ecotoxicity of SW underscore the need for caution when extrapolating findings to real wastewater matrices.

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4.14.P-Tu370 Ecotoxicological Response of Lichens to Pharmaceuticals in the Terrestrial Environment

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Environmental pollution with pharmaceutical preparations, which are necessary for the maintenance of human and animal health, is becoming one of the most important environmental problems. Currently, many pharmaceutical active substances are used for the treatment or prevention of diseases in humans and animals. Due to their widespread use and persistence, antibiotics have become an important group of emerging contaminants that are increasingly being studied. The ecological risks posed by antibiotics are a growing concern and require increasing research attention. However, the prevalence of veterinary antibiotics in the environment and their effects on the environment are still poorly studied. The study aimed to evaluate the ecotoxicological effects of antibiotics on non-target organisms - lichens. Toxicity of tetracycline (TC), ciprofloxacin (CIP) and sulfamethoxazole (SMX) was tested based on physiological and biochemical lichen responses. The study results showed that mycobiont and photobiont reacted to different antibiotics indicating dose- and time-dependent toxicity. The study highlighted the response of symbiotic organisms to exposure to toxic environments.

4.14.P-Tu371 Assessing Environmental and Endocrine Risks of Diclofenac Liposome Encapsulation and Its Byproducts

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The increase of pharmaceuticals in the environment, mainly due to inadequate disposal and inefficient removal in wastewater treatment plants, has raised concerns about their potential effects on ecosystems and human health. To approach this problem, new methodologies, such as the encapsulation of compounds that allow a more controlled release at the site of action, are being developed to reduce their environmental impact across their life cycle. This study aimed to evaluate the effects of a novel liposomal encapsulation of diclofenac, as well as the impact of production residues, namely diafiltration waste, on different organisms, while also testing a commercial diclofenac solution for comparison. To this end, an acute toxicity test with *Aliivibrio fischeri* (Microtox), a Yeast Estrogen Screen Assay (YES) and an acute immobilisation test with *Daphnia magna* (OECD 202), were conducted. Microtox results demonstrated effects on the bioluminescence produced by *A. fischeri* in all samples, with the final product, diafiltrated liposomes loaded with diclofenac, being the one with the highest effect, with an EC50=3.45mg/L. In agreement with this, the results obtained for *D. magna* demonstrated lower survival rates from the diafiltrated sample with an EC50=26.16mg/L. On the other hand, the commercial diclofenac solution induced the lowest effects in both organisms, with an EC50=15.09mg/L and EC50=144.32mg/L, for *A. fischeri* and *D. magna*, respectively. The results from the YES assay suggested that the final product and the placebo liposomes may have potentially induced endocrine disruption, as they were able to bind to the estrogen receptor in the yeast. Even though this new method may solve a current problem, in this case, liposomal encapsulation of diclofenac showed higher toxicity than the commercial formulation, suggesting that the process may affect the targeted and non-targeted delivery of the compound.

4.14.P-Tu372 Developments Towards a Marine Environmental Quality Standard (EQS) for Diclofenac

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Diclofenac is a nonsteroidal anti-inflammatory human and veterinary medicine widely detected in European surface waters. Proposed Environmental Quality Standards (EQS) which include an assessment of toxicity to aquatic organisms have been proposed by the European Commission (EC) to support the aims of the Water Framework Directive (WFD) (EC 2022).

As part of the development of the chronic ecotoxicity dataset for diclofenac, originally intended to support this EQS derivation, a programme of new testing was established. This comprised a series of new GLP studies using freshwater and marine invertebrates with primary objectives of filling clear taxonomic gaps in the long-term aquatic effects dataset for diclofenac.

The marine element of this testing programme comprised four GLP studies, covering the effects on diclofenac on the reproductive performance of three echinoderm species (two sea urchins *Paracentrotus lividus*, *Psammechinus miliaris* and a starfish *Asterias rubens*) and a polychaete annelid (lugworm *Arenicola marina*). The sea urchin studies investigated the effect of diclofenac on the fertilisation of embryos and their subsequent development, following an acute exposure of adult animals immediately prior to spawning of gametes, while the remaining studies focused on fertilisation only. All four studies were performed at the Scymaris laboratory in Brixham, UK and were conducted according to the principles of Good Laboratory Practice. The detailed test parameters and results of these four studies will be presented in the poster.

In addition, we will consider an updated reliable and relevant long-term marine ecotoxicity dataset for diclofenac including these new data, and propose potential approaches to developing a marine EQS which focuses specifically on effects in saltwater organisms. Finally, the outcomes will be compared with the marine EQS proposed by the EC (based on effects in freshwater organisms) and discuss the level of protection afforded by the different EQS.

4.14.P-Tu373 A Framework for Interdisciplinary Environmental Risk Assessments of Antibiotics: From Patient to the Environment

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Benign by design concept promotes reducing the adverse impacts of chemicals from the beginning of their initial design stages. In light of this concept, the European Union project TransPharm (grant agreement number: 101057816) is dedicated to developing greener pharmaceutical molecules and their production processes to foster towards a more sustainable European pharmacy sector. In this study, we developed a framework for an interdisciplinary environmental risk assessment (ERA) to evaluate the influence of fluoroquinolones (FQs) currently on the market, as well as their derivatives developed within TransPharm, on the aquatic environment. This framework integrates both experimental methods, including bacterial growth inhibitory test and cyanobacteria toxicity test, and modelling tools such as the physiologically based pharmacokinetic model PK-Sim and probabilistic risk assessment. It aims to combine various aspects associating with the fate of antibiotics, such as antibiotic efficacy, pharmacokinetics in the human body, degradation pathways influencing persistence, with ecotoxicology, to offer an approach for selecting pharmaceuticals that can bridge human health benefits and environmental sustainability. Our results indicate that higher degradability enabled the FQ-derivative ciprofloxacin-hemi (CIP-hemi) to show a lower environmental risk quotient (RQ) in surface waters than ciprofloxacin (CIP) when prescribed at the same dosage level. In addition, intravenous administration was predicted to result in a lower risk compared to oral administration. However, the antibiotic efficacy of the derivative restricts its applicability. Although the RQ for CIP-hemi remained consistently below the risk threshold, it could completely achieve efficacy criteria for *Escherichia coli* ATCC 25922 only. For other strains tested, efficacy criteria was reached at relatively high dosages ≥ 400 mg every 12 hours.

4.14.P-Tu374 Improving Monitoring and Environmental Risk Assessment of Pharmaceuticals, Antimicrobial Resistance and Pathogens from Terrestrial to Aquatic Environments

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Global contamination of soil and aquatic ecosystems by pharmaceutical and microbiological pollutants (such as antimicrobial-resistant microorganisms and/or pathogens) raises severe concerns about impacts on ecosystem health and repercussions on humans and animals. Preserving ecosystems from adverse ecotoxicological effects of pharmaceuticals and their transformation products and limiting the environmental spread of antimicrobial resistance and pathogens is imperative to reach several UN Sustainable Development Goals as well as the European Green Deal, Water Framework Directive and Biodiversity Strategy for 2030. To meet these huge environmental and societal challenges, public and private stakeholders and EU policy makers require improved monitoring and Environmental Risk Assessment to protect microbial diversity and functions in contaminated ecosystems. In this context, the MSCA Doctoral Network Pharm-ERA funded by the European Union (Grant Agreement 101119261) proposes a high-level interdisciplinary and intersectoral research and training network based on 10 doctoral projects covering scientific disciplines including environmental and analytical chemistry, microbial ecology, ecotoxicology, molecular biology (including multi-omics approaches) and chemical fate/effect modelling. Pharm-ERA involves 9 Beneficiaries (including 2 non-academics) and 6 Associated Partners (including 5 non-academics), committed to contribute to research, training, dissemination, communication and exploitation of results targeting end-users such as Environmental consultancies and agencies. Pharm-ERA will provide EU with high-level scientific experts who will further shape and implement the next generation of environmental management strategies, EU guidelines and regulations to reduce the adverse environmental effects and risks of pharmaceuticals and the spread of antimicrobial resistance and pathogens in terrestrial and aquatic environments. This will ensure sustainability of our ecosystems, fostering positive impacts on human and animal health and well-being far beyond the Pharm-ERA project.

4.14.P-Tu375 Assessing Reliability and Applicability of In Silico Tools for Chronic Aquatic Ecotoxicity of Human Pharmaceuticals

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Residues of active pharmaceutical ingredients (APIs) and their metabolites have been detected in surface waters, raising increasing environmental concerns over time. Since 2006, the European Medicine Agency (EMA) requires an environmental risk assessment (ERA) for marketing authorisations of new medicinal products for human use. An ERA typically requires three experimental No Observed Effect Concentrations (NOECs) or EC10 values, i.e. for freshwater algae, invertebrates (typically daphnids), and fish. The problem is that many APIs authorized before 2006, often referred to as legacy APIs, lack sufficient ecotoxicity data. Generating these data is widely considered unfeasible due to ethical, technical, and financial constraints. In silico tools offer a promising alternative, by predicting ecotoxicity data in a way that saves time, reduces costs, and minimizes animal testing in line with the 3Rs principles (Replacement, Reduction, and Refinement of animal studies).

In this study, we identified existing in silico tools that can be used to predict chronic ecotoxicity endpoints in fish, Daphnia, and algae for 1,637 pharmaceuticals. In addition, we compiled an overview of chronic experimental data, including regulatory-compliant NOEC and EC10 for human pharmaceuticals. Predicted No-Effect Concentrations (PNECs) were calculated using both in silico and experimental ecotoxicity data. Predicted and empirical chronic ecotoxicity data, as well as PNECs, were compared in order to evaluate the performance of the selected models.

The preliminary results indicate that some tools have moderate predictive potential. However, the models typically fail to predict ecotoxicity values that fall at the extremes, particularly the very low ecotoxicity values. This is concerning in the ERA context because overlooking these effects may result in underestimation of environmental risks. To address this, we have started exploring whether the accuracy of predictions could improve within specific ranges of toxicokinetic and physicochemical parameters, such as logD or molecular weight. By exploring these factors, we want to gain a clearer understanding of the conditions in which the predictive models can be confidently used, particularly within the ERA context. In parallel, we aim to identify situations where the predictions may be less reliable and should be treated with caution.

4.14.P-Tu376 Utilising Optimised Machine Learning Soil and Sediment Sorption Models for Pharmaceutical Mobility Classification

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Active pharmaceutical ingredients (API) have been widely detected in the environment, with concerns raised due to their potential for toxicity. API mobility within the environment has therefore become an increasingly important aspect of a chemicals hazard assessment, with an understanding of sorption behaviour in different solid environmental matrices a key part of this. Existing sorption modelling approaches for APIs are reliant on traditional regression-based methods for predicting sorption, which are limited in their ability to incorporate important descriptors, particularly those relevant to the ionisation of APIs in different environments. Machine learning (ML) has been presented as an avenue to improve prediction of complex sorption behaviours, with random forests showing good performance for soils and sediments. This work aimed to develop and evaluate new random forest sorption models for APIs, using freely available molecular descriptors to increase the accessibility of ML approaches for predicting sorption. New models were retrained using high quality soil and sediment linear sorption coefficients, with descriptors iteratively removed based on cross-validation to maintain model performance while reducing required data inputs. Validation of the optimised model against two external datasets from literature and industry demonstrated good performance, with 88.31% and 69.67% of predictions within a factor of 10 of experimental observations respectively. This optimised model was then used to assess the mobility of 1515 human and 146 veterinary APIs in use in Europe. Linear sorption coefficients for these APIs were predicted across 7 OECD-recommended soil types, normalised to organic carbon, and assessed against new EU chemical mobility thresholds. Based on model predictions 14.39% of human and 18.49% of veterinary APIs would be classified as very mobile in at least one of the 7 soils. Variability between soil predictions suggests that environmental pH is an important consideration for assessing ionisable pharmaceutical mobility. The results of this work highlight the advantages of ML as a tool for predicting API sorption behaviour across different environments, especially for legacy APIs where reliable empirical data is often limited, and serves as an example of how increasing accessibility to more advanced modelling approaches can provide a valuable way to prioritise APIs for further hazard assessment.

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4.14.P-Tu377 Prioritising Pharmaceuticals by Comparing Plasma Concentrations Predicted by a Fish PBK Model with Human Therapeutic Plasma Concentrations

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Environmental risk assessment of pharmaceuticals faces challenges due to limited ecotoxicological data, compounded by the costs and ethical considerations of generating new data. Consequently, risk evaluations often rely on PNECs derived from incomplete datasets or QSAR predictions, underscoring the need for targeted prioritization of high-risk compounds for further testing.

4.14.P-Tu378 Quantification of the Potential Hazard and Risk of Pharmaceutical and Personal Care Products in Wastewater Treatment Plants, Combined with Hazard and Risk Screening Using In Silico Approaches

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Pharmaceuticals and personal care products (PPCPs) are contaminants of emerging concern. After wastewater discharge, PPCPs can reach various aquatic environments, including drinking water, where they may pose risks to human health and wildlife. Understanding the removal efficiency (RE) of these substances in wastewater treatment plants (WWTPs), and their subsequent environmental fate and behaviour, is a priority for the scientific and regulatory communities. This study examines the potential Persistence, Mobility, Bioaccumulation and Toxicity (PMT and PBT properties) of more than 200 PPCPs found in WWTPs worldwide and integrates experimental RE data, quantified in different WWTPs, with predictions from in silico models based on Quantitative Structure-Activity Relationships (QSARs). A dataset was compiled from 32 literature studies collecting information on WWTPs in Europe, Asia, Americas, Africa and Oceania. Experimental data included PPCPs concentration in water measured from influents and effluents of more than 70 WWTPs, RE values, and treatment types. Ultimately, data for 245 chemicals were selected for further analysis. The study included RE assessment, PBT and PMT

assessment using in silico predictions and the assessment of the potential risk in the aquatic environment. The molecular structures of the studied chemicals were represented using Simplified Molecular Input Line Entry System (SMILES) strings, which were used as input to apply freely available QSARs (e.g., OECD Toolbox, OPERA, EPI Suite, and QSAR-ME Profiler). After verifying the applicability domain of these models, predictions were compared with regulatory thresholds to identify hazardous chemicals and to screen PMT and PBT substances. Sixteen out of 245 chemicals were identified as potential PBT or PMT substances, six of which may pose a potential risk based on their concentration in WWTPs effluents reported in the literature. This study provides valuable insights to manage potentially hazardous chemicals and to mitigate potential risks by enhancing WWTPs efficiency or by substituting problematic substances with alternatives that are more easily removed and/or do not possess PMT and PBT characteristics. Combining literature data with in silico predictions supports the effective assessment of substances that are not adequately removed in WWTPs.

4.14.P-Tu379 PECsw Refinement Approaches in Medical Products with No Well-Defined Treatment Regimen

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The Environmental Risk Assessment (ERA), defined by the recently-revised guideline (2024), is the evaluation of the environmental impact of Human Medicinal Product (HMP), based on its release in the environment after its therapeutic use by the patients. The ERA is mandatory for all new Marketing Authorization (MA) applications for medicinal products. Nevertheless, its conclusions and implications currently do not impede the MA approval of the HMP. Altogether, this guideline describes a step-wise procedure based on the evaluation of the environmental exposure of the drug product, considering the predicted worst-case scenario use (dosage and posology). ERA phase I includes the calculation of the Predicted environmental concentration for surface water (PECSW) of the drug product, considering its maximum daily dose and standard values of wastewater/inhabitant/day and fraction of treated population. If the obtained PECSW is higher than 0.1 µg/L, this non-acceptable risk leads to Phase II triggering. In Phase II, PECSW may be refined by modifying the fraction of market penetration (FPEN), the proportion of the population being treated daily with a specific drug substance, based on actual prevalence data of the disease/s in the target area, and/or on the worst-case treatment regimen/s. However, challenging are the cases of sporadically used HMP, for example in the context of surgery interventions, which have a supposedly unpredictable frequency and involve an unforeseeable number of patients. Additionally, for a drug that treats symptomatology (rather than a pathology with a well-defined dose regimen), the duration of the treatment is often only vaguely suggested in the package leaflet and shall be often adjusted based on patients' real needs. These risk assessments have to be adjusted based on alternative approaches, like reasoning by absurdity or definition of a likely posology even for symptomatology-addressed drugs. Even if these approaches are in line with the scope of ERA, they are not taken into consideration in the reference guideline. It is unequivocal that the lack of specific guideline indications for these cases implies the need for clarification from health authorities in order to harmonize the approaches used for these peculiar assessments. Moreover, it is desirable that the Authorities responsible for the evaluation of the ERA assessments are willing to accept alternative approaches, adequately justified, in line with the scope of the ERA.

4.14.P-Tu380 Assessing the Value of Including Sediments in OECD 106 Studies for Pharmaceutical Environmental Risk Assessments

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Environmental risk assessment (ERA) of human medicines is typically focussed on the specific exposure pathways associated with down the drain chemicals, i.e. those that enter domestic wastewater treatment plants and subsequently enter the environment, either to surface waters or to the terrestrial environment via application of sewage sludge to land. The target environmental compartment for a drug ultimately depends on the partitioning behaviour between aqueous and solid media, firstly during wastewater treatment and then between surface water and sediment (for aqueous emissions) and/or between soil and porewater (for terrestrial emissions). The new European Medicines Agency (EMA) guideline on environmental risk assessment of human medicines requires an OECD 106 test for adsorption to sludge and soils. The role of sediment testing is less clear, although in both the previous (2006) and current (2024) EMA ERA Guideline, no preference is given to the use of soils or sediments in the OECD 106 sorption test. Because of this, many OECD 106 studies have included sediments, given that partitioning coefficients derived using sediments are arguably more relevant than those derived using soils for calculating potential sediment exposure

To explore the value or otherwise of including sediments in the OECD 106 study, historical adsorption data on pharmaceuticals have been reviewed for their utility under the new EMA ERA Guideline. Specifically, this work explores the importance of organic carbon vs other drivers of partitioning of pharmaceuticals, whether soil and sediment data can be pooled for the purpose of calculating geometric means, the implications for calculation of the predicted environmental concentration in sediment (PEC_{sediment}) and the determination of a meaningful predicted no effect concentration in sediment (PNEC_{sediment}), and if, or when, corrections for organic carbon content are appropriate. The hope is that this work will provide guidance on the use of adsorption data for soils and sediments, and address whether there is value in including sediments in future OECD 106 studies for pharmaceutical ERAs.

4.14.P-Tu382 Implementation Details Matter: Defining Micropollutants and their Hazardousness in the Context of the European Union's Revised Urban Wastewater Treatment Directive

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As a part of the European Union's recently Revised Urban Wastewater Treatment Directive (UWWTD), the pharmaceuticals and cosmetics industry sectors will be responsible to pay extended producer responsibility (EPR) fees to fund quaternary treatment of micropollutants, where micropollutants are substances, including their breakdown products, that are usually present in the aquatic environment, urban wastewater or sludge and that can be considered hazardous, even at low concentrations, to the environment or human health on the basis of the relevant criteria set out under the regulation on classification, labelling and packaging (CLP Regulation). Small producers are exempt from EPR fees, and large producers are expected to pay based on the quantity and hazardousness of the micropollutants in products they place on the EU market. However, both the definition of a micropollutant and the basis for determining its hazardousness lack clarity, which is operationally critical to successfully implementing the EPR under the UWWTD.

We provide a review of scientific bases for defining micropollutants and determining their hazardousness. Considerations include, but are not limited to, the relevant health and environmental hazard categories under the CLP Regulation, biodegradability evaluation schemes, and data on treatment efficiency and the fate of substances in wastewater treatment plants. We then explore potential operational definitions and approaches, which may be considered in the context of the UWWTD.

The outcomes and recommendations from this study are intended to support identifying a scientifically sound process for regulatory implementation of the UWWTD by the EU member states. Hence, we also discuss the practical considerations and implications of the possible definitions of micropollutants and of the approaches to determine their hazardousness, with the goal of supporting a fair, harmonized and transparent application of the polluter pays principle and ultimately influencing innovation to identify and transition to the use of more benign substitutes for the micropollutants in question wherever possible.

4.14.P-Tu383 Using a Consistent Framework for Literature Reviews Improves Confidence in Environmental Risk Assessment of Pharmaceuticals

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There is growing interest and openness to incorporate new approach methods (NAMs) into regulatory environmental risk assessments (ERAs) to minimize traditional reliance on animal test methods and utilize existing data on chemical properties and environmental fate. The European Medical Agency's (EMA) 2024 Guideline on ERA of Human Medicinal Products allows the use of literature studies as alternatives to requisite empirical studies and recommends the use of approaches to minimize the need for new animal testing. However, there is limited guidance on how to conduct the literature data search, potentially leading to multiple Applicants conducting ERAs for the same Active Pharmaceutical Ingredient (API) using inconsistent data, especially if not all relevant data is identified or assessed for relevance and reliability. Furthermore, the available data will be presented in different formats, making it difficult for the regulators to check gaps and inconsistencies in the datasets.

A framework, presented separately, has been developed to identify critical studies/data and to assess their relevance and reliability in a robust and consistent manner, so they can replace and/or supplement empirical studies required by the regulations. Several case study examples will be presented for the application of the proposed framework for literature search and review strategy, including for a new API antibody drug conjugate and a generic API with multiple published studies. The case study examples will

highlight the decision points, where using an explicit framework for selecting and evaluating studies to include in the ERA supports consistency and reliability.

The initial case study examples demonstrate how a consistent approach to literature review and data searches can not only increase the confidence in the regulatory ERA's reliance on existing data, but can also avoid unnecessary and redundant testing, particularly animal testing. The findings and lessons learned will contribute to establishing transparent use of existing relevant literature studies and to promoting the 3Rs principles, in accordance with the stated intention of the 2024 EMA Guideline on ERA.

4.14.P-Tu384 A Standard Approach for Literature Data Review Required Under Revised EU Pharmaceutical Environmental Risk Assessment Guidance

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The European Medicines Agency (EMA) recently updated guidelines for the environmental risk assessment (ERA) of medicinal products for human use (EMA 2024). This guideline now requires the applicant to provide a complete literature review (including details on how the literature search was performed, i.e. identity of the search engine and terms used for the search), with the stated purpose to identify endpoints of significance to the ERA and to prevent repetition of (animal) studies. However, no further instructions are provided on how to conduct the literature search. In this poster, we present an approach for the literature and data search required for regulatory pharmaceutical ERAs. Case-studies on application of this approach are presented separately. The approach is based on established principles and strategies to promote and maintain consistency among regulators and applicants.

For both new and existing active pharmaceutical ingredients (APIs), a bibliographic search is performed to identify data from published literature. Searches can be conducted on one or more platforms (e.g., Web of Science, Scopus, PubMed, US EPA ECOTOX), using a tiered process to balance the scope of the search and amount of information to assess. The first tier is based on the substance identifiers only. A second tier utilises search strings specific to information types (i.e. physchem, fate, ecotox, bioaccumulation etc.), if refinement is required to reduce the number of results to screen. A set of criteria is proposed to determine potential relevance to endpoints of significance to regulatory ERA.

For existing APIs, authoritative databases are searched for any proprietary data and existing approaches to ERA, e.g., assessment reports and summaries from the EMA, US FDA, Fass, and ECHA CHEM databases/websites.

Any potentially relevant data identified this way is assessed in detail to determine the ultimate relevance and reliability for inclusion in ERAs, following Criteria for Reporting and Evaluating ecotoxicity Data (CRED, Moermond et al. 2016) for ecotoxicity studies or equivalent criteria (e.g., OECD test guidelines) for other study types (e.g., physico-chemical and environmental fate data).

We propose this approach as a standard for literature searches for APIs requiring a regulatory ERA, which benefits both, the applicant and the reviewer, by ensuring that relevant and reliable literature data is used to support regulatory ERAs.

4.14.P-Tu385 Applicability of the European Commission's framework on safe and sustainable by design to the pharmaceutical sector

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The chemical sector contributes significantly to anthropogenic impacts on planetary health. Thus, there is a need for a green transformation. The same holds for the pharmaceutical sector. To assist with this transformation, European Commission's Joint Research Centre (JRC) developed a framework on Safe and Sustainable by Design (SSbD) for chemicals and materials (SSbD framework). The general structure of the SSbD framework leaves room for sector-specific priorities and practices. This study explores the applicability of the SSbD framework to the pharmaceutical sector; more specifically to the development, production and use of Human Medicinal Products (HMPs). We show that the stage-gate process in R&D of HMPs fits very well with the design assessment process of the SSbD framework, making the framework conceptually applicable to pharmaceutical R&D.

Future efforts should focus on the development of i) methods to predict environmental safety and sustainability based on the limited data available during R&D, ii) a pragmatic procedure integrating SSbD into HMPs innovation, and iii) a weighing system considering also environmental safety and sustainability parameters alongside patient safety and medical efficacy. Although the assessment phase of the JRC s SSbD framework has specifically been developed for innovation purposes, we propose an expansion of its scope to pharmaceutical products already on the market. The reason is its applicability by healthcare actors to guide safer and more sustainable choices regarding the use of marketed HMPs. We call this approach Safe and Sustainable by Comparison (SSbC) and show that the assessment principles of the SSbD framework can be applied to SSbC.

4.14.P-Tu386 Pan-European Risk of in-use Pharmaceuticals to the Environment

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Pharmaceutical pollution is of rising concern due to the increasing demand and use of human and veterinary medicines and their subsequent release into the environment via excretion, improper disposal and manufacturing. There is growing scientific literature on the occurrence and fate of active pharmaceutical ingredients (APIs) in aquatic and terrestrial environments, and vast amounts of existing monitoring data available across Europe, particularly for surface waters. Using available data, our work aims to assess pollution risk of APIs to aquatic and, where possible, to terrestrial ecosystems. As part of the EU HORIZON ETERNAL project which studies aspects of sustainable API manufacture, use and disposal, our work designs a ranking approach to identify current in-use APIs that present the greatest risk to aquatic and terrestrial environments. A database has been constructed which collates available API exposure monitoring datasets across environmental compartments (i.e., surface waters, groundwaters, sediments and soils) from numerous data portals (i.e., Environment Agency water quality monitoring, UK Water Industry Research Chemical Investigation Program, NAIADES, NORMAN EXPODAT, German Environment Agency UBA pharmaceutical database) and scientific literature. In total, our database contains concentrations for > 600 pharmaceutical compounds (parent and transformation products) from monitoring sites across Europe (> 30 countries), which we have used to explore the magnitude and frequency of occurrence of API exposure. Some of the most commonly measured APIs in the environment include antibiotics, analgesic and estrogenic medications, and some of the highest exposure concentrations collated from the data sources are those for antibiotics and veterinary insecticide substances. To catalogue the relative toxicity of APIs detected in the environment, the hazardous concentration for 50% of species (HC50) values from Species Sensitivity Distributions (SSDs) were collated from the National Institute for Public Health and the Environment SSD database for 12,386 chemicals. Our community-based risk assessment approach uses SSD-derived HC50 values as these give a lower uncertainty and higher robustness in comparison to traditionally used HC5 values. Environmental exposure concentrations and SSD-derived HC50 values for ~400 API parent and transformation products are combined to provide a risk ranking of current in-use pharmaceuticals to ecosystems.

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4.14.P-Tu387 Exploring the Trade-Off Between Environmental Impact and Therapeutic Effect. A cross sectional study assessing preferences among Swedish general practitioners

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Introduction/Aim: Prescribing and use of pharmaceuticals in primary care belong to the most important factors contributing to healthcare s environmental impact. Consequently, prescribers are major stakeholders that can reduce the environmental footprint of pharmaceuticals. There is limited research on general practitioners (GPs) attitudes towards environmentally responsible prescribing practices. This study explores Swedish GPs preparedness to incorporate environmental considerations into treatment decisions. **Methods:** A questionnaire to assess environmental considerations in prescribing was developed and distributed to 1,233 Swedish GPs and physicians under training between September 2023, and June 2024. The questionnaire included three simulated patient cases, focusing on pharmaceuticals used for contraception, pain management, and blood pressure reduction, to assess the trade-off between environmental impact and therapeutic effect of the treatment. Additional questions about the respondent s attitudes coupled to pharmaceuticals and environmental issues were included. Responses were analysed

using descriptive and inferential statistics.

Results: Out of 1,233 GPs invited, 272 responded (response rate: 22%). More than half (58%) were women, 63% were born in 1980 or later, and 29% were physicians under training. A majority (88%) indicated that environmental and climate considerations were very or fairly important. Many of the respondents were willing to prescribe a less effective pharmaceutical if it was environmentally preferable, 77% for pain management and blood pressure reduction and 50% for contraception. Environmental impact was ranked as the least important factor compared to treatment cost, regional treatment guidelines, dosage intervals, and user-friendliness in terms of factors influencing their prescribing decisions. However, 24% of respondents stated that they often or always considered environmental impact when prescribing pharmaceuticals.

Conclusions: Swedish GPs are willing to consider environmental impact when making prescribing decisions for certain conditions, but other factors are valued higher in their decision-making.

4.14.P-Tu388 Unnecessary Fish Bioaccumulation Testing of Pharmaceuticals Driven by Use of Ion-Corrected LogDow in PBT Assessment

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The recently revised EMA guideline on environmental risk assessment of human pharmaceuticals provides guidance specific to ionizable compounds for the assessment of fish bioaccumulation potential as part of the PBT assessment. Two physical chemical properties inform this:

- 1) Dissociation constant (pKa) value(s) experimentally determined according to OECD TG 112,
- 2) LogDow data, experimentally determined according to OECD TG 107 or 123, "... determined as a function of pH covering an environmentally relevant pH-range (at least 3 pH values ranging from pH 5 to 9), e.g. by measuring the pH-lipophilicity profile (log D as function of pH)".

In addition to this, an "ion-corrected logDow for the neutral molecule" is to be determined mathematically according to equation $Kow = Dow \cdot (1 + 10^{(pH - pKa)})$. PBT/vPvB assessment is triggered if any extrapolated, ion-corrected Dow value(s) meet or exceed criteria ($\log Kow > 4.5$), irrespective of the pH at which the molecule is predicted to present as the neutral species. PBT screening initially starts with assessment of the bioaccumulation criteria by an experimental fish BCF test. The guidance further clarifies "...the BCF test [OECD305] should be conducted at a stable pH consistent with the most bioaccumulative form of the test chemical (usually the non-dissociated form or the form with the most neutral molecule species)."

However, fish testing triggered by a corrected Log Dow manifest at a $pH < 6$ or > 8.5 would not be possible to conduct due to the restrictions of the OECD 305 test guideline. Some extension beyond this recommended pH range may be possible but great care should be taken to account for fish welfare and the physiologically tolerable range of species tested when interpreting results.

PBT/vPvB assessment is often described as an assessment of the "intrinsic" properties of a molecule, intended to account for the potential for harm irrespective of exposure. However in the EMA guidance there is no clarification on the environmental relevance of the pKa value used to determine the ion-corrected LogDow; therefore even at very extreme pH's a fish BCF study could be triggered which has no environmental relevance.

Here we will present a scientific approach to consider the environmental relevance of a PBT assessment, demonstrating both theoretically and using relevant examples, that the experimentally determined LogDow values are sufficient for the assessment of bioaccumulation, and avoid unnecessary fish testing.

4.14.P-Tu389 Concept for Implementation of an Environmental Information, Classification and Dissemination System in Germany ("Arzneimittelindex Umwelt")

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Residues from pharmaceuticals enter the environment via various entry pathways and are measured in some areas at concentrations exceeding threshold concentrations. Effects on organisms can be detected and with the increasing use of pharmaceuticals in an ageing society, this problem will become even more acute in the future. There are several initiatives along the pharmaceutical life cycle that try to tackle this problem. One promising way of lowering the impact of pharmaceuticals on the environment is

encouraging physicians and pharmacists to prescribe and dispense medicines in an environmentally responsible manner. The aim of this study was to develop a concept for an environmental information, classification and mediation system suitable for the German healthcare system. Furthermore, this concept should be developed on the basis of lessons learnt from these pioneer countries and in close coordination with relevant stakeholder groups to reach high effectiveness and acceptance. To achieve this goal, case studies from Sweden, Finland and Scotland were combined with a literature review of peer-reviewed literature, policy briefs and grey literature, supplemented by interviews with relevant local stakeholders. Based on this information a concept suitable for the German healthcare system was developed and discussed in stakeholder workshops

The concept of this information and classification system is feasible in Germany and is able to lower the entry and impact of pharmaceuticals into the environment (PiE) at the point of decision making. Over and above that, several other stakeholder such as health insurances can use this information to make environmentally sound decisions. This study is relevant, as it describes a concrete concept ready to be implemented in a time where PiE are of rising concern. Additionally, in contrast to end-of-pipe approaches it displays a possible solution approach that can be implemented easier, faster and more cost effective. Furthermore, examples from other countries show, that such a system is effective in driving decisions and is therefore an effective way of environmental decision making in drug prescribing and dispensing.

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4.14.P-Tu390 Revision of the Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use

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The European Medicines Agency (EMA) has published a revised version of the guideline (GL) on the environmental risk assessment (ERA) of medicinal products for human use. First published in 2006, the GL describes the assessment of the potential environmental risks and hazards of human medicinal products (HMP) arising from their use. It provides guidance for applicants and regulators on how the ERA should be performed. After releasing a draft revision of the GL for public consultation in 2018, extensive feedback was received after the six-month consultation period. The ERA drafting group who undertook the initial draft revision worked through these comments between 2019 and 2024 and made additional amendments to the published draft to address this feedback. This drafting group comprises technical and regulatory experts recruited from national competent authorities (NCAs) and national environmental authorities and works under the remit of the Nonclinical Working Party of the CHMP. Responding to the feedback received during the public consultation, the revised GL aims to deliver the guidance in a more detailed, comprehensive, and visually appealing way. Regulatory and technical aspects addressed in the final revision were issues regarding clarification of scope concerning excipients, access to existing ERA, especially with regard to generics, the need for a deeper explanation of secondary poisoning (SP) assessment, and the persistence, bioaccumulation and toxicity (PBT/vPvB) assessment. Furthermore, adjustments were made in Phase II Tier B risk assessment regarding the refinement options of the predicted environmental concentration of the surface water compartment (PEC_{sw}) and concerning entry pathways of HMP into the groundwater compartment. Finally, changes were implemented regarding the proposed labelling for PBT/vPvB substances and a manual has been included as an annex which describes in detail the Simple Treat model used in the Phase II Tier B risk assessment to refine the PEC_{sw}. The revised GL provides a more comprehensive approach to the ERA both for applicants and assessors, taking into account scientific developments and changes in relevant guidance documents under other legislative frameworks.

4.14.P-Tu391 How Doctors and Pharmacists Can Avoid Harmful Medicines – A Simple Concept to Flag Environmentally Friendly Substances

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Pharmaceuticals are released into the environment through multiple pathways and can be found in significant concentrations, already exceeding threshold concentrations in some areas. There are numerous ongoing initiatives along the pharmaceutical lifecycle trying to reduce the input of pharmaceuticals into the environment. A high impact in this respect can be achieved at the beginning of the life cycle, as well as by reducing the number of prescriptions/dispensing of medicines or optimising them towards eco-

prescriptions/dispensing. An environmental information and classification system for pharmaceuticals, such as in Sweden and Finland, is a tool that can help choose more environmentally friendly pharmaceuticals. In a feasibility study the implementation of such a pharmaceutical index environment (Arzneimittelindex Umwelt) in Germany was investigated. In addition, to the pillars information and dissemination systems a concept for the classification of active substances in pharmaceuticals was requested, which enables doctors and pharmacists to distinguish between environmentally harmful pharmaceuticals from those available to them.

Several existing classification concepts are based on different approaches or combinations of approaches: effects on non-target organisms, fate in the environment (persistence), consumption, occurrence in the environment or carbon footprints. After consultation with potential users in the healthcare system, a simple and understandable system such as the embrytox.de labelling is proposed, which should be based on independently verified results. For the information system the use of public results from European environmental risk assessments according to the technical guideline of the European Medicines Agency is suggested. The same criteria could also be used to classify all marketed active substances. In that way the classification approach will cover the environmental risks and hazards identified in study reports used in the application process, as well as the chemical nature of the substance.

We will present the concept of a simple, clear and concise classification system by flagging substances that are less harmful or unlikely to pose a risk to the environment but also harmful substances. The poster will include an impact assessment of the proposed classification scheme for all active substances marketed in Germany and will also show practical application examples for doctors and pharmacists.

4.14.P-Tu392 Monograph System for Active Substances of Pharmaceuticals

Jan Pridohl, Daniela Gildemeister and Arne Hein, German Environment Agency (UBA), Germany

Since the mid-2000s, Environmental Risk Assessments (ERAs) for active substances of human and veterinary medicinal products (HMP/VMP) have been required according to the respective EU Directives (2001/82 and 2001/83).

For a few years now, the data derived from those ERAs have been summarised in (European) Public Assessment Reports ((E)PAR) after the authorisation procedure of the respective medicinal products. These documents are only partially publicly available and usually lack several environmental information e.g. data already investigated through other authorisation procedures. In some cases, information is not published due to the authorisation holder's confidentiality rights. Therefore, it is hardly possible to collect all relevant data for an active substance based on (E)PARs, only. Also, updates of (E)PARs are currently not legally required. Thus, harmonised evaluations of HMP and VMP, respectively, containing the same active substance are rather impeded.

Generating an active substance based monograph system would be a promising solution and would also be in accordance with the international and EU environmental information law (environmental information according to Art. 2 (3) (b) of the Aarhus Convention), as well as the EU Commission's proposal for a revised Pharma legislation (COM [2023] 192 final).

Such a monograph system should contain not only fate and effect data, but also predicted environmental concentrations (PEC) and necessary risk mitigation measures (RMM). When all information are then summarised in one document, it will be easier to update the monograph with the latest data. Different levels of access to the data can be provided for the relevant stakeholders.

The poster will present our proposal for a monograph structure of ERA data. Verified as well as publicly available data for a well known active substance of a pharmaceutical over the counter product have been summarised. The poster includes endpoints (e.g. PEC, effect and fate data), a proposal for a study summary and a list of implemented RMM.

A substance monograph should be frequently updated by the (Co) Rapporteur/Reference Member State. This monograph approach also supports the data sharing and data provision recommendation of the new ERA-Guideline for HMP (EMA/CHMP/SWP/4447/00 Rev. 1 - Corr.) and easily harmonises the EU-wide environmental risk assessment of pharmaceuticals.

4.14.P-Tu393 Availability, Need and Use of Environmental Data – State of the Art and Prospects for the Future

Daniela Gildemeister, Arne Hein, Jan Pridohl, Louis-Marvin Sander and Kim Teppe, German Environment Agency (UBA), Germany

"Environmental data are a prerequisite for identifying active pharmaceutical substances for which appropriate risk mitigation measures may be necessary. Formally, environmental data should be published according to the Aarhus Convention, but pharmaceutical data from authorisation procedures lack this transparency.

In this presentation we want to show what is already available and what could be available in the future

with respect to the proposal of the European Commission (EUCOM) for a revised Pharmaceuticals Legislation. In reference to the Aarhus convention there may be possibilities to publish more data than it is currently the case.

Therefore, a review is done of current legislation and recent interpretations by a German administrative court (Cologne) about the confidentiality of environmental data. The new GL was analysed for publication issues and the legal restrictions within this GL were identified. Published data available in (European) Public Assessment Reports (EPAR) were compared with available data from authorisation processes to identify publication gaps. The available data were compared to the number of active substances on the market in Germany and the number of monitored substances to identify the availability gap.

In the proposal of the EU Com for a revised Pharmaceuticals Legislation the main changes foreseen to improve the data availability situation are

4.14.P-Tu394 Out of sight – The Insufficiency of Terrestrial Veterinary Pharmaceutical Regulatory Limits in Early Risk Assessment

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The VICH GL 6 outlines a tiered assessment scheme that is mandatory for all active substances (AS) used in veterinary medicines before they enter the market. As the first step, the predicted environmental concentration of the AS in question is compared to a so-called action limit of 100 µg/kg for soil. If this action limit is exceeded, an extended environmental risk assessment is required. This limit is currently based on data that were recorded between 1973 and 1997 in the USA. Since then, new active ingredients with higher efficacy (and, therefore, potential environmental impacts at lower concentrations) have been developed and put on the market. This consequently elevates the probability of environmental and organismic impact, which in turn affects biodiversity and, ultimately, the natural functioning of ecosystems.

A critical evaluation of the action limit is therefore necessary. Does it still serve its purpose as a precautionary decision criterion on whether an experimental Phase II risk assessment must be conducted? To assess the protectiveness of the soil action limit of 100 µg/kg, we evaluated 82 tests (34 plant and 48 earthworm tests) for 18 parasiticides, 28 antibiotic and 5 other AS, using data from European Medicines Agencies Public Assessment Reports, supplemented by internal data of the German Environment Agency. We included parasiticides in the data evaluation, although the action limit does not apply here, as their environmental hazard is determined by their toxicity to insects. Tests between model predictions reveal no difference between models with and without parasiticides (with parasiticides $n = 51$, without parasiticides $n = 33$). For each AS, we included the lowest available NOEC/EC10 and fitted a sigmoidal non-linear least squares model in the range of [0,1].

18±5 % of the NOECs/EC10 values are below 100 µg/kg soil. This reduces to 17±6 % if only non-parasiticides are included in the data analysis. A total of 11 substances are below or equal to the action limit, 7 antibiotics and 4 parasiticides. In order to ensure that the action limit covers approximately 95 % of AS currently on the market, a reduction from 100 to 5 µg/kg would be necessary. The analysis shows that the current action limit is insufficient to protect organisms and ecosystems. In future revisions of the guideline, it will be necessary to adapt the action limit to current scientific standards.

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4.14.P-Tu395 Toxicity of the Veterinary Pharmaceuticals Pyrantel Pamoate and Ractopamine in Zebrafish Embryos (*Danio rerio*)

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Widespread use of veterinary pharmaceuticals to treat livestock has resulted in their entry into aquatic environments. Unlike human drugs, which pass through wastewater treatment plants before being discharged into the environment, veterinary drugs are usually excreted through urine and feces and released directly into the environment, potentially reaching rivers, ponds, etc. The presence of veterinary drugs in water bodies is a current issue of concern, as their excessive use is a potential risk to aquatic organisms. Two widely used veterinary drugs are pyrantel pamoate and ractopamine. Pyrantel pamoate is an antiparasitic used to treat infections by intestinal worms such as pinworms, roundworms, and

hookworms; it acts as a depolarizing neuromuscular blocking agent, causing a sudden contraction, followed by paralysis, of the worms. Ractopamine is a synthetic β -adrenergic agonist (β -adrenergic receptors modulate metabolic functions in fish such as oxygen uptake, heart rate, vascular resistance, and the binding affinity between hemoglobin and oxygen), used as a feed additive to increase feed efficiency, accelerating growth and increasing weight gain in animals. Using zebrafish as a model teleost, the goal of this study was to characterize the toxicity of pyrantel and ractopamine using an extended zebrafish embryo toxicity test OECD 236). Embryos were microinjected with either DMSO or with pyrantel or ractopamine at 0.4, 2.0, 10, and 50 ng/g of egg and reared until 15 days post-fertilization, during which time they were assessed for mortality, malformations, heart rate, and locomotor disorders. There was a dose-dependent increase in mortality (EC = 36,982 ng/g egg). There was a small but statistically significant dose-dependent increase in heart rate between control and pyrantel-treated (2, 10, and 50 mg/mL) embryos at 48 h, with control embryos averaging 160 bpm, whereas pyrantel-treated embryos averaged 162 (0.4 mg/mL), 164 (10 mg/mL), and 166 (2 and 50 mg/mL) bpm at 48 hpf. This is expected to impact the larvae's locomotor activity (behavior) directly and may be affected and neurodevelopmental disorders may be identified in the drugs. The effects of ractopamine are currently being determined. Overall, results to date suggest that embryonic exposure to pyrantel might have posed a threat to fish and contributed to a better understanding of potential environmental hazards posed by some veterinary drugs in fish.

4.14.P-Tu396 Using Zebrafish G Protein-Coupled Receptors to Obtain a Better Appreciation of the Impact of Pharmaceuticals in Wastewater to Fish

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Pharmaceutical discharge to the environment is of concern due to its potential adverse effects on aquatic species. It is estimated that around 40% of pharmaceuticals target G protein-coupled receptors (GPCRs). The *in vitro* transforming growth factor- β (TGF β) shedding assay has been applied to measure the antagonistic activities of pharmaceuticals against human GPCRs. However, their ability to stimulate fish GPCRs remains unclear. Here, the antagonistic activities of 30 pharmaceuticals against zebrafish dopamine (zD2a, and zD2c), adrenergic family member (z β 1), and histamine (zH1 and zH3) receptors were measured by the TGF β shedding assay. The study found interspecies difference in binding affinities between human and zebrafish: pharmaceuticals more strongly inhibited the zebrafish zD2c and zH1 receptors than human D2 (hD2) and hH1 receptors, while zebrafish zD2a and z β 1 were less inhibited than human hD2 and h β 1 receptors. The interspecies difference in binding affinity for hydroxyzine (an antihistamine medication) was explained by molecular docking. Pharmaceutical potency against zebrafish GPCRs and predicted effluent concentrations were used to predict equivalent quantities (EQs), and then these EQs were used to prioritize pharmaceuticals of concern in wastewater in England and Japan. This study highlights the use of the TGF β shedding assay adopting zebrafish GPCRs to better understand the ecological effects of pharmaceuticals on fish.

4.14.P-Tu397 Ecological Risk Assessment of Sulfa Drugs: A Case Study Using Component Based Approach for Chemical Mixtures

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Sulfa drugs are a large group of substances widely used as human and veterinary medicines. They are very similar in structure and have antibacterial activity through the same mode of action. In addition, they are harmful to aquatic organisms and thus, an ecological risk assessment has been carried out. In Japan, environmental risk assessments (ERAs) for regulatory purposes focus primarily on individual chemicals. However, substances with the same mode of action are expected to have combined effects when present in the environment at the same time. It is therefore necessary to assess the combined effects of the sulfa drugs rather than the individual chemicals.

The aim of this study is to verify and further investigate relative potency factor (RPF) approach for higher tier ecological combined risk assessment that we have been exploring so far, using simple estimated environmental discharge values in addition to actual measured concentrations in the environment, with a focus on the use of sulfa drugs in livestock and aquaculture.

Literature searches were carried out to collect environmental concentration data using databases such as J Dream III. Some of the literature we obtained reported that water concentrations of some sulfa drugs and trimethoprim (TMP) were relatively high in some rivers near livestock farms. The combination of

sulfamethoxazole (SMX) and TMP has been reported to have a synergistic antibacterial effect by inhibiting successive steps in the bacterial folate synthesis pathway. Based on the above, the assessment group in this study was refined to seven sulfa drugs used in veterinary medicine and feed additives and TMP. A regression equation derived from trend analysis between parameters related to the mode of action and toxicity values was used to calculate the RPF of the sulfa drugs included in the assessment group. Although the synergistic effect of TMP on the effects of sulfa drugs is not considered in this study, the inclusion of synergistic effects is a common issue in the combined effect assessment of substances acting on target molecules. We also report on a case study of ecological combined risk assessment, where the concentration in rivers was estimated from the amount of antibacterial substances used in the livestock and fisheries industries, and it was used as the exposure concentration.

4.14.P-Tu398 Determination of the Effect of the Presence of Coccidiostats and Nutritional Metals on the Degradation of Tiamulin in Poultry Litter

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The potential impact of feed additives on the degradation of veterinary medicines in poultry litter is not well understood. Currently guidance provided by the European Medicines Agency and the European Food Safety Authority states that only one substance should be assessed at a time in a simulated manure degradation experiment. By focusing on a single substance, the current assessment of veterinary medicines and feed additives does not consider the impact of co-contaminants on the degradation of these compounds in manure. Manure is a sink for many contaminants in the environment, therefore it is critical to ensure that the risk assessment processes are environmentally realistic. A series of experiments were designed to explore the impact of co-contaminants frequently detected in manure, namely coccidiostats (both ionophores and synthetic) and nutritional metals on the degradation of the antibiotic tiamulin. Litter was collected from a working poultry farm and physico-chemical properties were characterised before use in series of manure degradation experiments. The litter was dosed with tiamulin at a concentration of 10 µg/g and different combinations of coccidiostats and nutritional metals including, salinomycin, diclazuril, copper and zinc. Spiked manure samples were incubated under controlled conditions to evaluate the impact on the fate of tiamulin during its degradation. Methods employed were in line with the harmonised experimental guidance published by the European Medicines agency in 2011 and OECD320 (where appropriate). This study is ongoing with preliminary results showing that the presence of synthetic coccidiostats may slow the degradation of tiamulin in poultry litter. The results from this study will allow us to better understand the factors that drive the variability in the degradation in veterinary medicines in different poultry litters from different farms and understand whether these co-contamination leads to an underestimation of exposure during environmental risk assessment.

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4.14.P-Tu399 Veterinary Antimicrobials in EU: Goals, Use and Ecotoxicity

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Antimicrobial use contributes not only to antimicrobial resistance, but poses environmental effects like ecotoxicity, altered species interaction and in general ecosystem health. Therefore, attention to the antibiotics use both for humans and food producing animals increases in scientific and political agenda. So far antimicrobial use for veterinary needs comprises the biggest share of all antimicrobials. Consequently, much tighter targets for reducing the use of veterinary antibiotics compared to the use for humans are set on EU level. Hence, paper aims to analyse antimicrobial use for veterinary antimicrobials in EU countries over 2011-2022 and estimate distance to target for 25 EU member states. In addition, potential ecotoxicity related to the veterinary antibiotics use is also estimated. Data of the European Surveillance of Veterinary Antimicrobial Consumption on overall sales of veterinary antimicrobial medicinal products and according to antimicrobial class (tetracycline, sulphonamides, fluoroquinolones, 3rd- and 4th-generation cephalosporins, other quinolones and polymyxins) is used for this purpose. Results show that over analysed period overall use of veterinary antibiotics has decreased, with the least drop of fluoroquinolones. However, further decrease is needed to reach EU target and proposed global levels for veterinary antimicrobials use. Despite decrease of overall and per PCU antimicrobials use, some countries are still using two or more times extra veterinary antimicrobials compared to the set goal for EU on average. Hence, in addition to the human health threats, those countries contribute relatively more to the environmental degradation, too. Therefore, such countries should rethink current policies or their

implementation to reach more sustainable use of veterinary antibiotics and to implement one health approach.

4.14.P-Tu400 Ecological Risk Assessment of Human Pharmaceuticals Detected in Japan: Seasonal and Temporal Variations

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Guidance for environmental risk assessment (ERA) of new human pharmaceuticals were published in 2016 by Japan Ministry of Health, Labour and Welfare but the risk assessment became voluntary-base and the comprehensive ecological risk of pharmaceuticals have not been conducted in Japan. Last year, we presented the results of short-term (sub-)chronic aquatic toxicity tests using three species, zebrafish (*Danio rerio*) short-term test with embryo and sac-fry stage (OECD TG212), *Ceriodaphnia dubia* reproduction test (USEPA's WET test method), and green alga (*Raphidocelis subcapitata*) growth inhibition test (OECD TG201) for nearly 30 out of approximately 100 pharmaceuticals detected in rivers (95 percentile values were used for the risk assessment) all over Japan. This year, we additionally conducted these three tests for 5 pharmaceuticals without chronic data to determine No Effect Concentration (NOEC). Since the total of nearly 700 water samples were collected for comprehensive chemical analysis of nearly 100 pharmaceuticals using an LC-MS-MS to determine the spatial and temporal distribution of measured environmental concentrations (MECs) in the period of 2019-2021 all over Japan. So, this year, we conducted ecological risk assessment to determine the distribution of risk quotient (MEC/NOEC) in Japan. We found that antibiotics such as macrolides are relatively at high risk with MEC/NOEC ratio above 0.1 for alga in some sites while the maximum ratio was in the range of 0.01-0.1 for daphnid. The risk ratio for fish was found to be below 0.001 for most of the water samples and least risk for the selected 100 pharmaceuticals but we need to keep in mind that the testing was the short-term testing at embryo and sac-fry stages and endocrine disrupting effects on reproduction were not included.

4.15.P Sunscreens and Personal Care Products in the Environment: New Data and Approaches to Evaluate Environmental Risks and Possible Solutions

4.15.P-Mo384 Monitoring and Effects of UV-Filters on Diverse Algal Species

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The widespread use of UV filters in personal care products (PCPs) has raised concerns about their potential environmental impact, particularly on aquatic ecosystems. This study investigated the presence and ecotoxicity of four UV filters homosalate, octocrylene, benzophenone-3, and bemotrizinol in UK rivers and their effects on 12 algal species. A monitoring campaign sampling water from 19 locations across 10 rivers in Yorkshire, UK, over eight months was also carried out. The ecotoxicity study employed a newly developed method using a well plate reader to assess algal growth inhibition. Twelve algal species, including green algae, blue-green algae, and diatoms, were exposed to seven concentrations of each UV filter for 72 hours. Through GC-MS, the samples were analysed for the various UV filters. The concentrations detected ranged from ng/L to µg/L with octocrylene exhibiting the highest concentration, peaking at 807 ng/L in the River Aire, while benzophenone-3 reached 430 ng/L in the River Wharfe. EC50s values were determined, revealing significant interspecies variability in sensitivity. The diatom *Aulacoseira granulata* was the most sensitive species, with EC50 values of 0.027 mg/L, 0.0061 mg/L, and 0.295 mg/L for homosalate, octocrylene, and benzophenone-3, respectively. In contrast, green algae exhibited higher EC50 values, often exceeding the water solubility of homosalate and octocrylene. This study highlights the presence of UV filters in riverine systems and their potential ecological risks. The findings suggest that *A. granulata*, not currently included in OECD guidelines, may be a critical indicator species for future ecotoxicity assessments. These results emphasize the need for comprehensive research into the cumulative effects of UV filters in aquatic environments.

4.15.P-Mo385 Occurrence and Distribution of UV Filters in Sediments from the Baltic Sea

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The occurrence of organic ultraviolet filters (UV filters) is of increasing concern due to their ability to be endocrine disrupters and bioaccumulative in the environment. Globally, the use of organic UV filters as

active ingredients in sunscreens, personal care products and industrial materials is growing exponentially. The Baltic Sea is a popular holiday and leisure region exposed to high anthropogenic pollution by persistent, toxic, and bioaccumulative pollutants. One group of these pollutants is UV filters, which enter the Baltic Sea mainly through river inflows, wastewater treatment plants, and recreational activities. Previous studies have highlighted the presence of UV filters in the coastal areas of the Baltic Sea. The goal of the project is to investigate the spatial distribution of UV filters in open seas and coastal waters, simultaneously also their distribution in sediments in the Baltic Sea. In the current study, sediment cores were collected from four sampling stations in the German Baltic Sea. The sediment cores were sliced every two centimeters and examined individually for UV filters. The advantage of this approach is that it allowed us to analyze undisturbed sediments and study the distribution of UV filters at various sediment depths. The extraction of UV filters from the sediment samples was carried out utilizing the QuEChERS method. The QuEChERS method was significantly optimized using suitable extraction agents with subsequent purification steps by solid phase extraction (SPE) to yield maximum recovery. The final extracts were further measured with LC-MS/MS. Our Initial findings detected UV filters, octocrylene, benzophenone-4, UV-P and PBSA in surface water samples. This study provides more insight if UV filters with high log Kow values, such as octocrylene, avobenzone, homosalate, and padimate-O, accumulate in the sediment due to their high lipophilicity and persistence. This research provides a more comprehensive understanding of the distribution of UV filters at different sediment depths in the Baltic Sea, which helps us examine the role of sediments as sinks for UV filters.

4.15.P-Mo386 Organic UV Filters Detected in Coastal Waters and in the Coral *Acropora cervicornis* on the Florida Reef Tract

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Organic UV filters have been detected in both seawater and in marine biota, highlighting the potential for uptake and environmental risk from exposure. However, a limited number of studies have measured UV filters in seawater near coral reefs, and even fewer have examined UV filters in coral tissues. This study was designed to examine correlations between UV filter concentration in the water column and parallel UV filter concentrations in tissues of the endangered Atlantic staghorn coral *Acropora cervicornis*.

The concentrations of 12 organic UV filters were assessed in matched seawater (shallow surface and at coral depth) and coral tissue samples from one offshore nursery in Fort Lauderdale, Florida, and two offshore nurseries in the Florida Keys. The onshore nursery at The Florida Aquarium (FLAQ) served as an ex-situ control. No UV filters were detected in either seawater or coral tissues at the ex-situ onshore control site, and some UV filters were either below the detection limit or detected in very low (ng/L) concentrations in seawater at the offshore nurseries. Six of the 12 UV filters were detected in shallow and coral-depth water at the offshore nurseries located near Fort Lauderdale and near Tavernier in the Florida Keys, and three of the 12 organic UV filters were detected in shallow and coral-depth water near Carysfort Reef in the Florida Keys. Octocrylene, avobenzone, and homosalate were detected at all three offshore nursery sites. In general, avobenzone, oxybenzone, and padimate were detected predominately in the dissolved fraction, and homosalate, octinoxate, and octisalate were detected only in the particulate fraction. Octocrylene was found in both fractions, with the highest concentrations at the northernmost (Fort Lauderdale) and southernmost (Tavernier) nurseries.

Overall, organic UV filter concentrations in the water column were consistent with expected levels of anthropogenic contamination at each site and temporal changes in rainfall patterns. These data inform the range of environmentally relevant concentrations for future risk assessments on the potential impacts of UV filters, and provide critical support for regulatory policies and management decisions concerning UV filters in Florida coastal waters. As UV filter concentrations are highly variable in space (location, water depth, distance from source) and time, further assessments are required to identify sources and the temporal range of concentrations near coral reefs.

4.15.P-Mo387 Dissipation Kinetics of Ultraviolet Filters in Mesocosms and Occurrence in a Freshwater Beach on the Canadian Prairies

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UV Filters (UVFs) are found in a wide range of cosmetics, personal care products, and commercial stabilizers. UVFs are known to be toxic to aquatic organisms in both freshwater and marine environments, and are also known to be strong endocrine disruptors, thereby impacting human and environmental health.

It is important to quantify the exposure to total UVFs in a recreational environment and to calculate aqueous dissipation kinetics and half-lives to elucidate environmental persistence and fate. We quantified four of the most popular and common UVFs approved for Canadian sunscreen and cosmetic usage in freshwater mesocosms in Winnipeg, Canada over summer/fall 2024. Data collected was used to backstop what we saw in a freshwater beach study conducted in June to September 2022, and to guide future full-scale experiments. The four UVFs were octisalate, octocrylene, oxybenzone, and sulisobenzene. The mesocosms were divided into two treatments in triplicates: sediment and macrophytes, and with water-only; and they were dosed with a mixture of the UVF suite at 50 µg L⁻¹ each. They were chosen to be representative of the range of hydrophobicity (LogKOW) of UVFs at 5.77, 7.35, 3.79, and 0.88, respectively. Analyses were done using direct injection, isotope dilution, ultra-high performance liquid chromatography-tandem mass spectrometry, and polarity switching; and aqueous dissipation half-lives were calculated. For sulisobenzene, the mesocosms with sediment and water-only treatments, half-lives of 17 and 30 days were calculated, respectively. Oxybenzone had lesser initial concentrations most likely due to some sorption to plants, sediments, or the tanks themselves, and in the sediment and water-only treatments half-lives of 2.9 and 3.6 days were calculated, respectively. Fluctuations in concentrations of UVFs are most likely due to user application choices in addition to sorption to sediments, photolysis, and hydrolysis which is what was seen in the freshwater beach study. Although oxybenzone was seen to be the most labile in the kinetics study, it was the most abundant UVF at the beach over the summer, which speaks to consumer usage. The impact of these results can guide water quality guidelines to protect ecosystem health, and to industry to drive new eco-friendly sunscreen formulations.

4.15.P-Mo388 Environmental Fate of UV Filters: Comparing Lake Monitoring Data with Controlled Exposure in Artificial Ponds

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In the EU 47 substances are currently authorised for the use as UV-filters in cosmetic products. Additionally, the inorganic and several of the organic substances listed in the EU Cosmetics Regulation are also registered or authorised for other uses (agrochemicals, biocides, coatings, inks, stabilisers, cleaning agents, air care) according to the REACH registration information and may thus enter the environment via additional pathways. Accordingly, these substances can enter the aquatic environment directly through bathing or via WWTP effluents.

The fate of the UV filters was investigated in five different lakes and four artificial ponds in Switzerland. After sampling, the organic UV filters were preconcentrated by Oasis HLB solid phase extraction and afterwards determined on a high-performance liquid chromatography coupled to triple quadrupole mass spectrometer. The studied UV filters were ingredients currently used in sunscreens which were as follows 2-Ethylhexylsalicylate, 2-Phenylbenzimidazole-5-sulphonic acid (PMDSA), Avobenzone (AB), Octinoxate, Octocrylene, Oxybenzone, Ethylhexyl triazone, 4-Methylbenzylidene camphor, Disodiumphenyldibenzimidazoltetrasulfonate, Isoamyl 4-Methoxycinnamate, and Homosalate.

Octocrylene occurred in almost all sampled lakes in concentrations of over 1 µg/L in summer. Concentrations of up to 5 µg/L were determined in the smaller bathing waters. High concentrations were found in the surface water and less in the water column. AB was determined in small lakes in concentrations of up to 3 µg/L and in at least 10 times higher concentrations in surface water than in the water column. However, the hydrophilic PMDSA was sometimes found in concentrations of up to 3.7 µg/L in the surface water, twice as high as in the water column. This suggests that the formulation of the sunscreen products is also influencing the behaviour of the UV filters in the water body.

4.15.P-Mo389 Freshwater Safety Assessment of UV-Filters Using a Spatially Resolved Aquatic Exposure Model for Down-the-Drain Substances in Europe

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A key aspect of environmental safety of consumer use down-the-drain (d-t-d) ingredients is environmental exposure in receiving waters. This is the reason for legislation such as the European Urban Wastewater Treatment Directive that was implemented to set standards for wastewater treatment to help reduce environmental loading of d-t-d ingredients to better protect human and environmental health. A global

framework to estimate d-t-d substance concentrations in river catchments across the globe that leverages large spatial datasets on population, wastewater treatment (WWT) infrastructure, per capita water use, river connectivity, and river flow has been developed and demonstrated for several countries to better assess exposure potential. This framework was built on the well-established iSTREEM® model for the United States, and provides estimated river concentration distributions based on spatial variability of these parameters, as well as chemical-specific properties such as WWT removal and in-stream decay. Recently, this framework has been expanded to include Europe by leveraging official European Union data on several WWT parameters, including geographic locations of wastewater treatment plants (WWTP), and demonstrated good agreement with reported values for Europe. The model is publicly available and free for use. We present the application of this model to two key UV-filters used in personal care products (PCPCs), Oxybenzone and Octinoxate, and compare to monitored concentrations. We also demonstrate the model application to freshwater safety assessment by comparison to predicted no-effect concentrations (PNECs). Modelled concentration distributions were found to be in good agreement with monitoring data, while still being conservative. Additionally, 50th, 75th, and 90th percentile modeled and monitored concentrations were found to be substantially lower than PNECs, respectively, indicating favourable margins of safety even at the high-end of the concentration distribution. The use of spatially resolved exposure models are key tools for understanding concentration distributions across modelled regions and for use in prospective safety assessments of substances that are released down-the-drain. They can also be valuable to more accurately prioritize ingredients that could be targeted for refined removal via wastewater treatment.

4.15.P-Mo390 Developing Reliable Standard Analytical Protocols for the Detection of Multiple Organic UV Filters in Environmental Matrices

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Ultraviolet (UV) filters are used in a wide range of products, including sunscreens, and have been detected in water, sediment and biota. Orders of magnitude different concentrations have been observed in environmentally matrices and in toxicity thresholds reported. Differences reported may in part be due to the varying analytical methods used. Furthermore, adequate and accurate assessments of the concentration of the UV filter under study in toxicity testing is critical and underpins the reliability and defensibility of the toxicity thresholds derived from the study. We have observed contamination from laboratory plastics, carry-over of UV filters if re-used containers were not adequately solvent cleaned/baked, losses during analytical sampling, storage, extraction and analysis at various points depending on the UV filter under study and the materials used. In a previous oxybenzone study we found losses due to binding to glass sampling containers, retainment in the solid-phase cartridge, which would have resulted in underestimations of toxicity if not accounted for. We summarize our lessons learnt and recommend appropriate extraction procedures and analytical assessments for working with UV filters from field-collected environmental matrices and toxicity test samples with the goal to develop standard methods for UV filters.

An LCMS-based analytical method was developed to detect and quantify twelve organic UV-filter compounds, with limits of detection ranging from 0.02 to 9 ng/L. In order to test the effectiveness of the extraction method and the utility of the recovery standards, spiked solutions of all analytes at two different concentrations were prepared in deionized water and artificial seawater (n=6). These samples were prepared, extracted, and analyzed using the same method as environmental samples. In addition, standard solutions in artificial sea water were prepared and kept in the dark at room temperature, 4 and -20°C before being analyzed in order to test the analytes stability. The use of isotopically-labeled recovery standards added before solid-phase extraction increased both recovery and reproducibility. All compounds tested had a recovery of 80% or greater after recovery standard correction. This indicates that the extraction and analytical methods are well-suited for the analysis of UV filters in seawater samples. Additionally, all UV filters were shown to be stable over short periods at all tested temperatures.

4.15.P-Mo391 Temporal and Spatial Variations of UV Filters at a Popular Recreational Beach in Florida, USA

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Ultraviolet filters are a diverse group of compounds with a wide range of commercial applications, including as ingredients in sunscreens. To understand the temporal variation in concentrations of UV filters seawater samples were collected over a diurnal period in March and May 2024 at a popular

recreational beach in Fort Lauderdale, Florida, USA which has coral reefs located offshore. Three composite samples (n=10 discrete samples) were collected along two 100m transect locations that were different distances from the shore (T1; nearshore in the bathing zone and T2 ~50m from the shoreline). Samples were immediately processed by filtering (GFF) and solid phase extraction to prepare the dissolved fractions with the GFF filters frozen and later extracted via hexane extraction to prepare the particulate fractions. During processing recovery standard spikes were added. All extracts were analyzed quantitatively for 12 organic UV filters using LC-MS.

Eight analytes, oxybenzone (BP-3), octocrylene (OC), homosalate, octisalate, avobenzone, octinoxate, and, OD-PABA were measured in all samples, with May levels consistently higher. UV filter concentrations were higher at T1 and reduced significantly at T2, with at least a 60% decrease. All UV filters followed similar temporal trends with peaks in the mid afternoon (2-4 pm) reflective of the number of recreational users. Partitioning of the UV filters between the dissolved and particulate fractions matched their physio-chemical properties. BP-3 concentrations were nearly all in the dissolved phase and only a small fraction was present in the fraction retained on the GFF-filters i.e., particulate phase. Alternatively higher concentrations of OC were observed in the particulate versus dissolved phases reflective of its lower solubility and higher logK_{ow}.

Overall, these results highlight that aquatic organisms in popular recreational areas may encounter UV filters that are highly variable over a 24-hour period rather than a steady environmental concentration, emphasizing the importance of sampling for UV filters at times of both high and low recreational activity. Concentrations rapidly decline out of the bathing zone highlighting that both location and time is important in risk assessments. Furthermore, to understand total concentrations and exposure routes and minimize analytical error, it is important to conduct assessment of partitioning by filtering samples.

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4.15.P-Mo392 Monitoring Campaign for Organic UV Absorbents from Recreational Sunscreens on Okinawa Beaches Using Passive Sampling with Alabaster-Cement Composite Disks, Grab Sampling, and a Questionnaire Survey

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Organic UV absorbents found in cosmetics, particularly in sunscreens, have been reported to pose potential ecological risks to aquatic organisms, including coral bleaching. In recent years, scientists worldwide have monitored concentrations of these compounds in beaches and coastal areas to assess exposure levels for ecological risk assessment. However, UV absorbent concentrations vary widely based on factors such as the number of swimmers, tides, sunscreen usage, and other environmental conditions. Therefore, determining both peak concentrations and time-weighted average concentrations (TWAc) is essential to evaluate acute and chronic risks. In this monitoring campaign, we investigated daily concentration trends of seven UV absorbents in beach water through grab sampling. We also used DGT® passive sampling with alabaster-cement composite disks to estimate TWAc in the water. Additionally, sunscreen types and quantities used by beachgoers were recorded through a questionnaire survey. The seven UV absorbents analyzed included benzophenone-3, EHMC, 2-phenyl-5-benzimidazole sulfonic acid, octyl dimethyl PABA, octocrylene, bemotrizinol, and ethylhexyl triazone, which were quantified using liquid chromatography-tandem mass spectrometry. Sampling was conducted at three sites: one within the beach area and two offshore. Approximately 200 tourists participated in the questionnaire survey. Concentrations of some UV absorbents fluctuated in response to the number of beachgoers throughout the day. However, benzophenone-3, which is no longer widely used in Japanese cosmetics, was not detected via grab sampling at any time. This monitoring campaign will continue over several years, providing valuable data to support ecological risk assessments and develop predictive models for UV absorbent contamination and its associated risks.

4.15.P-Mo393 Organic UV Filters in Irish Sediments and Biosolids

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The assessment and monitoring of ultraviolet filters (UVFs) in environmental matrices is of vital importance. Ongoing monitoring of UVFs restricted under the Cosmetics Products Regulation (CPR) is needed to ensure levels in the environment remains or falls below thresholds for high ecotoxicological risk. Investigation into emerging UVFs of concern, such as those listed under the Water Framework

Directive s (WFD) Watch List , is necessary where insufficient data is available to determine the safe levels of use. Meanwhile, determining the potential sources of environmental contamination with UVFs is needed to identify areas where preventative measures to reduce said contamination might be necessary. To that end, we report herein the results of a nationwide campaign which investigated levels of five prominent UVFs (4-MBC, BP-3, EHMC, HMS, and OC) in inland and transitional sediments across Ireland, along with sewage sludge/biosolids emanating from wastewater treatment plants.

Mean concentrations in biosolids were as follows: OC (666 ng/g); HMS (453 ng/g); 4-MBC (13.9 ng/g); EHMC (12.7 ng/g); and BP-3 (1.28 ng/g). Concentrations in Irish biosolids are lower than those determined in similar studies worldwide, though this is the first available data on HMS in biosolids. Mean concentrations in sediments were as follows: OC (3.66 ng/g); HMS (2.35 ng/g); 4-MBC (1.87 ng/g); BP-3 (0.19 ng/g); and EHMC (0.04 ng/g), showing a similar profile to concentrations detected in sediments. Though detected at comparatively moderate concentrations, 4-MBC was detected by far the most frequently: in 93 % of sediments samples. Ninety-fifth percentile risk quotients (RQ95) were determined for each UVF in sediment based on predicted no-effect concentrations as promulgated by the NORMAN Network. Each of 4-MBC, EHMC, and HMS were found to be of a moderate level of risk (0.40, 0.13, and 0.11 respectively) based on their measured concentrations. Significantly higher concentrations of UVFs were detected directly adjacent to or immediately downstream (<0.5 km) wastewater emission points ($p < 0.01$).

Continued monitoring of HMS and 4-MBC in the short term is recommended to determine the efficacy of regulations limiting their use, and further data is needed for OC, EHMC, and BP-3 to provide a more thorough risk assessment as pertains to the WFD. The elevated levels of HMS in biosolids found herein highlights the need for further assessment internationally.

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4.15.P-Mo394 The Ecotoxicological Effects of Two Contrasting UV Filters Derived from Sunscreens (oxybenzone & nano titanium dioxide), on the Temperate Marine Microalgae Species *Isochrysis Galbana*.

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Ultraviolet (UV) filters are organic and inorganic compounds added to sunscreens to protect against the sun's UVA and UVB rays. UV filters have been detected in both freshwater and marine matrices around the world, but despite the ubiquity of these compounds, little research has focused on the molecular, cellular and individual level effects on temperate marine organisms and primary producers.

Adopting a multidisciplinary approach, this research aims to understand the ecotoxicological effects of two widely used and contrasting UV filters, oxybenzone and nano-titanium dioxide on the temperate marine microalgae species *Isochrysis galbana*. Methods used include the use of flow cytometry to assess for cellular density and growth, chlorophyll fluorescence analysis, as well as oxidative damage and antioxidant response microplate assays. Additionally, biogeochemical techniques have been adopted to analyse the effect of UV filters on sulphur compound production using gas chromatography.

To date, our results indicate that environmentally relevant concentrations of oxybenzone reported in the literature (1-100 $\mu\text{g/L}$) can have adverse effects on *Isochrysis galbana*, with a significant decline in cell density within 24 hours of exposure at very low concentrations (1-10 $\mu\text{g/L}$) and mortality within 48 hours of exposure to 30-100 $\mu\text{g/L}$. Chlorophyll-a content within the algae significantly declined in a dose-response manner over 96 hours, but there was no indication of oxidative stress. For nTiO₂, there was no effect to the algae at 1 $\mu\text{g/L}$, but a decline in cell density and growth was observed between 10-100 $\mu\text{g/L}$ and 5-100 mg/L. Chlorophyll-a content was also affected from 10 $\mu\text{g/L}$, which continued in a dose-response manner. Lipid peroxidation was observed at both 50 and 100 mg/L of nTiO₂, and at these concentrations, 100% mortality took place within 72 hours.

This ongoing research aims to better understand the effects of both organic and inorganic UV filters on temperate marine organisms. Current regulations on the use of UV filters in personal care products in both the UK and the EU are lax in comparison to other global nations. This research aims to provide evidence that can help policymakers to understand the true potential of UV filters derived from sunscreens on

temperate marine organisms, and to make informed decisions about which compounds should be better regulated within sunscreens and personal care products.

4.15.P-Mo395 The Hidden Impact of Human Presence: Organic UV Filter Levels on an Oceanic Island Coastal Waters, Northeast Atlantic

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Subtropical oceanic islands have become increasingly popular tourist destinations. Official recommendations to protect the skin from harmful sun rays have led to a rise in the use of sunscreens containing UV filters, which are directly introduced into coastal waters during recreational activities. Characterized by high photostability and lipophilicity, organic UV filters are persistent compounds that can remain in the ecosystem for extended periods.

This study aims to evaluate the impact of human activities on coastal ecosystems, focusing on quantitatively assessing organic UV filters throughout the marine food web. By analyzing species across different trophic levels, including macro-algae, zooplankton, invertebrates, and fishes. We determined how these contaminants move through the ecosystem and asses their concentration in Madeira Island, Portugal.

Samples were collected from three sites: Funchal, the capital (densely populated and touristic, high anthropogenic pressure), Lombada dos Marinheiros in the west of the island (remote area, low anthropogenic pressure), and the Desertas Islands (uninhabited and Marine Protected Area). UV filters were extracted using Microwave-Assisted Extraction and analysed via UHPLC-MS/MS.

Results showed that organic UV filters' highest concentrations and detection frequency were from samples collected in Funchal, while the lowest levels were found in the Desertas Islands. Zooplankton and algae exhibited the highest concentrations, reaching up to 1028.6 ng/g and 292.67 ng/g respectively. Among fish, cowfish (*Sarpa salpa*) showed higher concentrations (up to 472.22 ng/g of total UV filter concentration), likely linked to herbivorous diet, while invertebrates (sea urchin *Arbacia lixula* and sea cucumber *Holoturia sanctori*) mostly had levels under the limit of detection, potentially due to low environmental exposure or dilution by ocean dynamics. Octocrylene and homosalate were the most common compounds, reflecting their prevalence in European sunscreen products.

These findings highlight the ecological impact of human activities on coastal waters, stressing the need for monitoring emerging contaminants to protect marine health and fish consumer's safety. The study underscores the urgency for standardized assessments of environmental levels and toxicological thresholds to inform effective mitigation strategies which could include European regulation to create and market more environmentally friendly cosmetics.

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4.15.P-Mo396 UV Filters Modify Light Colour Preference in the Zooplanktonic Species *Daphnia magna*

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UV filters are ubiquitous in the environment, having been detected globally in wastewater, freshwater and marine environments. While the use of UV filters in sunscreens and cosmetics are important for human health, their risk and impact to aquatic or terrestrial organisms is not well studied. Most ecotoxicological studies conducted so far with UV filters in aquatic invertebrates and algae used conventional endpoints such as mortality, growth and reproduction. There is less information on the effects of UV filters on zooplankton species. Many freshwater and marine zooplankton species show anti-predatory behavioural responses to light to avoid visual predators like fish. Antipredator behavioural responses include scape

responses to light such as increase motility under light and diel vertical migration characterized by swimming towards deep and dark waters during daylight and towards surface waters during night to feed on alga. It is also known that many of those species used particular light wavelengths to trigger their migratory movements and other ones to search for food. One of the properties of UV screens is to filter certain light wavelengths. Here we present data showing that two well known UV filters, octocrylene and benzophenone-3 are able to modify the light wave length colour preference of the zooplanktonic and ecotoxicological model species *Daphnia magna*. To do that we build a new behavioural device able to monitor vertical and horizontal locomotion tracks of organisms across varying light wave lengths and intensities. Our results indicate that sublethal concentrations of octocrylene were able to reduce the swimming speed of exposed organisms and increased their photophobia to the tested light wavelengths. Furthermore the blue colour preference of exposed organisms also changed: octocrylene at low concentrations decreased *D. magna* preference for blue light, whereas at higher concentrations together with benzophenone-3 increased the preference for the blue relative to the red, white and green lights. The above-mentioned light colour preferences changes may have important ecological effects as the blue colour light is the wave length that penetrates more into the water column, is the preferred one for several zooplanktonic species and is the one that trigger the most the diel vertical migration. Acknowledgement - The authors thank the Spanish Government for the funding of the projects PID2023-148502OB-C2, PID2023-151815OB-I0.

4.15.P-Mo397 A New Approach to Assessing the Impact of Active Ingredients and Cosmetic Formulations on Coral Reefs

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Threatened first and foremost by rising sea temperatures, pollution and ocean acidification, coral reefs are also undermined by human activities, which are accompanied by the introduction of cosmetic products into the marine environment.

The proposed protocol gathers coral cuttings from 12 to 50 coral species exposed to the product to be tested. After a 24-hour exposure period, the short-term impact is evaluated from observations such as polyp closure, loss of color, and signs of necrosis. The corals are then transferred to quarantine aquariums for a two-week recovery period to assess medium-term effects. Throughout the testing period, marine biologists closely monitor the corals health and physio-chemical parameters in the aquariums.

At first, a sunscreen formulation containing widely used organic filters was tested according to this protocol. After 20 hours of exposure, and the 14-day recovery period, 36% of the species displayed necrosis.

Replication of this test on more than 20 cosmetic formulae coupled with the analysis of the water in contact with the formulae resulted in the identification of key formulation criteria to characterize ecotoxic criteria.

This first cartography will also help us to select the most relevant species to establish EC50 and toxicity thresholds, in order to improve our understanding of species-specific sensitivities.

These results underscore the importance of comprehensive ecotoxicological testing of cosmetic products on a diverse range of coral species to accurately assess their potential impact on coral reef health.

4.15.P-Mo398 Advances in Standardized Ecotoxicological Testing with Scleractinian Coral Larvae

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Coral reefs face substantial ecosystem shifts under increasing anthropogenic threats. Personal care products, for example, are expected to contain compounds (e.g. UV filters) that may harm corals. To assess the effects of these compounds on corals, it is necessary to develop standardized test guidelines. Current methods mainly focus on the adult life stage of corals, however, juvenile corals and particularly coral larvae might be more sensitive to various stressors (e.g. substances, environmental samples). To close their lifecycle, coral larvae need to settle on the reef and develop into adult coral colonies. If the settlement process is inhibited, the swimming larvae will eventually die, which could have far-reaching consequences for the propagation of corals resulting in little or no recruitment of juvenile corals and large-scale reef degradation in the long term.

This study refines the experimental design for a standardized toxicity test on coral larvae, evaluating the larval endpoints settlement and mortality, which was first introduced by Miller and colleagues in 2022. To improve variabilities of recovery rates, the experiments here were conducted in glass containers, resulting in nearly 100% recovery rates of both, benzophenone-3 (BP-3) and the positive control Diuron (DCMU), which would allow to refer the determined EC50 or LC50 values to the nominal test concentrations. Preliminary results already indicated that the effect of BP-3 on the ability of larvae to settle may be the most sensitive endpoint for corals. However, implementing a settlement endpoint is challenging, as the application of biological settlement cues (i.e., crustose coralline algae and microbial biofilms) tend to (1) trigger variable settlement responses in different coral species and (2) the additional biological components in the test vessels may interact with the test substances. The recently described chemical settlement cue cycloprodigiosin reduces the variability in the settlement endpoint and prevents unintended interactions between the biological cue and the target substance resulting again in increased recovery rates. The latter improvements in the experimental design will enable further testing of the effects on coral larvae and develop holistic coral bioassays in a standardized way.

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4.15.P-Mo399 Improving the Accuracy of ex-situ Coral Growth Measurements using 3D-Scanning

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Growth measurements in scleractinian corals are important to monitor and evaluate various physiological processes. However, this has been somewhat challenging, especially for small colonies, fragments and over short time periods. This is often the case in laboratory ecotoxicological studies where precise size measurements are essential to reliably detect detrimental effects at high sensitivity.

Common methods for measuring coral size include (1) buoyant weight determination and (2) the evaluation of their footprint (shadow area) from a top-down view. Both methods have notable limitations: (1) buoyant weight measurements can be affected by setup variability, such as minor changes in water temperature or salinity, and the growth of non-target organisms (e.g., algae). (2) Footprint measurements, on the other hand, avoid these issues but may underestimate the complex three-dimensional (3D) growth of corals. However, recent advances in reliable 3D-scanning technologies offer the potential for consistent size measurements with increased accuracy.

In this study, we tracked the growth of three coral species (*Montipora digitata*, *Acropora austera* and *Porites cylindrica*) in an aquarium facility over three months, using buoyant weight, footprint area, and 3D-scanning methods. Small fragments of the coral species were characterized by different growth forms: *M. digitata* spread encrustingly across hard substrates, *A. austera* grew in complex shapes along a primary axis and *P. cylindrica* grew as relatively massive digitate branches. Growth rates were calculated for each species, and the accuracy and correlations among the methods were analyzed. Compared to 3D-scans, we found that buoyant weight measurements showed higher variability, while footprint area measurements were less accurate for corals with more complex growth forms. For encrusting coral fragments, such as *M. digitata*, footprint area measurements were highly correlated to 3D surface area determinations.

Our findings demonstrate that 3D-scanning offers the highest accuracy of the three tested measurements for coral growth. The evaluation of the footprint area can serve as a precise proxy for less complex growth forms (i.e., *M. digitata*). Using these improved methods enhances the reliability and sensitivity of growth measurements, paving the way for the use of coral growth as a sub-lethal endpoint in standardized chronic ecotoxicological testing.

Disclaimer/Disclosure: The authors declare that this study received funding from BASF SE (Ludwigshafen, Germany) through the grant Toxicity tests with corals and coral reef communities, grant number 5551622859. Opinions expressed in the paper are those of the authors; the funder had no role in the study design, data collection, analysis or interpretation of the study findings.

4.15.P-Mo400 Octocrylene Shows Ultimate Inherent Biodegradation in OECD Screening Tests

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Octocrylene (CAS 6197-30-4) is a widely used organic compound employed as a UV-B filter in

sunscreens, cosmetics, and various home care products due to its ability to absorb ultraviolet radiation mainly in the UV-B area. In cosmetic formulations, the liquid and oil soluble Octocrylene is not only valued for its photostability and good formulation properties but also for the ability to stabilise Avobenzone, an important UV-A filter. Octocrylene is also used to enhance UV stability in plastics, coatings, and textiles. Concerns regarding its environmental impact have emerged. In the EU, Octocrylene is listed on the Community rolling action plan (CoRAP) as suspected PBT/vPvB.

The REACH-Dossier for Octocrylene concludes the substance to be poorly biodegradable as under test conditions no biodegradation observed. Two studies for ready biodegradability are listed: OECD301F (28d, 1991) & EU Method C.4-D (28d, 1995).

We have studied the biodegradation behaviour of Octocrylene in different tests. We have conducted OECD301 (ready biodegradation, prolonged to 90d), OECD302 (inherent biodegradation, prolonged to 90d) and ISO 14852 (indication for biodegradation, 180d). In our tests, Octocrylene showed biodegradation in all tested systems. A certain lag time was observed until biodegradation started. Result of >70% in OECD302B after 28d allows conclusion as ultimate inherent biodegradable. Values further increase over 90d test time. OECD301B show 16% (30d), 70% (60d) & 75% (90d). Repetitions with different inoculum showed comparable results.

We therefore propose to change the biodegradability assessment of Octocrylene to Ultimate Inherent Biodegradable. We continue to evaluate the environmental performance and re-assess the exposure of Octocrylene in ongoing studies.

It is noteworthy that biodegradation results and lag times match the early 1990s results. OECD301 studies have a longer lag time and exponential growth is observed after the 28d window. OECD302 show similar end results, have shorter lag times and reach plateau faster.

4.15.P-Mo401 Environmental Fate, Hazard and Risk Assessment of the Ultraviolet Filter Bemotrizinol in Water and Soil

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Ultraviolet (UV) filters are essential active ingredients in sunscreens. They provide critical protection against harmful UV radiation and reduce the risk of skin cancer and premature aging. Ideally, effective sun protection should be achieved while ensuring that the UV filters are also safe for ecosystems. In this study, we evaluated the environmental fate, hazards, and risks associated with Bemotrizinol (BEMT), a broad-spectrum UV filter widely used in sunscreens to protect against both UVA and UVB radiation. Our assessment combined a comprehensive, and high-quality experimental data set with in-silico predictions, covering BEMT's environmental fate, transport, exposure, and toxicity.

Our results show that BEMT exhibits a strong affinity for soil and sediment particles due to its high hydrophobicity, resulting in significant adsorption onto organic matter. This property enhances its removal by sludge in wastewater treatment plants and minimizes its mobility in soil, thereby reducing the likelihood of leaching into groundwater or spreading across environments. In aquatic ecosystems, BEMT tends to associate with suspended particulates, further limiting its bioavailability in the water column. Additionally, its low aqueous solubility further mitigates the risk of significant exposure to aquatic organisms.

Our analysis also shows that despite its hydrophobic nature, BEMT has a low potential for bioaccumulation in aquatic organisms, likely due to its large molecular weight. Ecotoxicological studies reveal that BEMT does not cause acute and chronic toxicity to aquatic and soil organisms, including fish, algae, invertebrates, and microbes. Even at concentrations significantly higher than the predicted environmental concentrations, BEMT shows no notable adverse effects (including sub-lethal effects) on these species, indicating no ecological hazard. In conclusion, even in a highly conservative scenario where potentially large amounts of BEMT may enter the environment, its low bioavailability and lack of toxicity ensure that it poses no significant environmental risk.

4.16.P Understanding, Detection, Monitoring, and Management of Harmful Algal Blooms (HABs) and Natural Toxins in the Environment

4.16.P-We401 Microcoleus as Toxic Benthic Mats on Different Bottom Substrates: Ecophysiology and Distribution

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Benthic cyanobacteria, notably Microcoleus, contribute to harmful algal blooms globally due to their toxin production. Their thriving in nutrient-poor freshwater environments presents significant environmental and public health challenges. In May 2023, we observed Microcoleus growing in a small tributary of the Virgin River near the Temple of Sinawava. Benthic mats were collected from six sampling points, three each, from rocks and sand substrates and collected rock and sand samples, along with downstream water

samples. We aim to understand how these organisms survive in low-nutrient environments, explore interactions between toxic and non-toxic cyanobacteria and other bacteria, and analyze benthic community diversity and growth facilitators using metagenomics. Additionally, we aim to understand the influence of various substrate types on benthic mat composition and growth. An LCMS/MS toxin measurement analysis revealed that all the benthic samples contained anatoxin-a (ATX) ($377.13 \pm 18.05 \mu\text{g/g}$) and dihydro anatoxin-a ($5.15 \pm 0.3 \mu\text{g/g}$). ATX ($0.377 \mu\text{g/L}$) was also present in water samples. Low chlorophyll-a levels and microscopy results suggest the toxin in the water comes from benthic sources, not pelagic algae. The initial metagenomic analysis found that cyanobacteria constituted the majority (>60%) of all benthic mat samples, predominantly belonging to the *Microcoleus* genus, with <5% attributed to eukaryotic algae. Upon resampling in the fall (October), we observed a complete shift in benthic algae composition, with no presence of *Microcoleus*. Additionally, no ATX was detected in the algal mats or water samples. We are also studying distinct *Microcoleus* strains isolated from same location to compare genotype variations between lab-cultured and environmental samples. Our study, the first to focus on *Microcoleus* in Zion National Park, reveals adaptive phosphorus acquisition strategies in nutrient-poor conditions. This strain is notably more toxic and contains unique thiamine biosynthesis genes absent in other toxic genomes studied in New Zealand. Supported by NSF funding, ongoing work includes genotypic analysis and microbial interaction studies to better understand *Microcoleus* proliferation and toxin production. Our research team is also collaborating with experts from New Zealand, Switzerland, the United States Geological Survey (USGS), and the U.S. Environmental Protection Agency (USEPA) to advance our understanding of this cyanobacterium.

4.16.P-We402 Temperature and Salinity Affect Growth and Toxin Production of Estuarine Cyanobacterium *Microcystis aeruginosa*

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Coastal ecosystems, which act as essential connectors between inland waters and ocean systems, are now encountering unparalleled challenges fueled by human activities and climate change. *Microcystis aeruginosa* is recognized as a harmful cyanobacterial species with its ability to produce microcystins (MCs) and its tendency to bloom in estuarine environments. Although previous research has shown the impact of individual environmental conditions on the growth or toxin production of *M. aeruginosa*, the possible interactive effects and resulting changes in its toxicity remain uncertain. In this study, we initially conducted an orthogonal growth experiment to evaluate the effects of variations in temperature, salinity, pH, and nutrient conditions. This was followed by a full-factorial growth experiment, with temperature and salinity as primary variables. We measured intracellular and extracellular MCs content, along with phycocyanin levels, during both exponential and stationary growth phases. Toxicity was assessed through mortality and swimming behavior in the harpacticoid copepod *Nitokra spinipes* and the calanoid copepod *Acartia tonsa*, both estuarine species. Results indicated that both growth and MCs production were significantly induced by increasing temperatures (15 to 28 °C), but were reduced with elevated salinity levels (8 to 16 ppt). Furthermore, cell density and growth rate showed a strong correlation with both intracellular and extracellular MCs levels. A significant interaction between temperature and salinity was detected, while no correlation was observed between intracellular MCs and phycocyanin levels. Lastly, exposure to *M. aeruginosa* led to reduced swimming speed, higher inactivity, and increased mortality in *A. tonsa* compared to the non-toxic *Rhodomonas salina*, while *N. spinipes* showed no sensitivity to *M. aeruginosa* at environmentally relevant concentrations. This study emphasizes the combined effects of temperature and salinity on *M. aeruginosa* growth and toxin production, shedding light on potential risks associated with future blooms under changing climate conditions.

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4.16.P-We403 Portable and Semi-Automated System for Paralytic Shellfish Toxins' Monitoring

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Seafood aquaculture is heavily impacted by toxic harmful algal blooms (HABs) yearly. Paralytic Shellfish Toxins (PSTs) are among the most dangerous families of toxins and frequently affect seafood aquaculture. In this work, we describe the development of a portable and semi-automated system for detecting PSTs in seawater, allowing tracking of the appearance and progression of PSTs-producing HABs before the

concentration in the seafood reaches regulatory level thresholds. We designed and optimised a modular portable system for real-time in situ implementation. This system requires minimum operator intervention before placing the sample for further processing and analysis. The sample is pumped through a microfluidic chip designed to accumulate and disrupt the cells. The chip features a serpentine channel and a chamber with pillars to confine glass microbeads. Under a portable bath sonication, cells are exposed to mechanical stress, promoting cell walls and membrane breaking and releasing cellular content, including intracellular toxins. The resulting sample is then filtered to remove the debris and encapsulated in microdroplets with a composite formed by a covalent organic polymer, TpPa-COOH and, gold nanostars (GNSs). The TpPa-COOH@GNSs composites serve two purposes: they are used to preconcentrate and trap the biotoxins and enhance the Raman signal of the biotoxin through surface-enhanced Raman scattering (SERS). The microdroplets are accumulated in a microfluidic cartridge, and the read-out is performed using a portable Raman system with a 785 nm laser line. Finally, an automated measurement and analysis is performed using ML/AI algorithms to estimate the PST concentration in the sample. Although the system is still being optimised and the full specifications are being prepared, our results indicate that it can detect nM concentration of PSTs in the microalgae extract from the seawater sample. This system aims to facilitate early warning of HABs, helping to prevent seafood contamination beyond acceptable limits for human consumption and providing a tool to ensure food safety, economic growth, and sustainability of the aquaculture sector.

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4.16.P-We404 A Comprehensive Approach to Saxitoxin Effects in *Daphnia magna*

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Harmful algal blooms and the toxins produced during these events are a human and environmental health concern that impact fresh and marine waters worldwide. Among them, saxitoxin and its derivatives are of particular interest as potent natural aquatic neurotoxins produced by freshwater and marine algae species. Saxitoxins adverse impacts to human health are well known which prompted the World Health Organization (WHO) to establish a safety guideline for the concentration of these toxins in recreational freshwaters of 30 µg L⁻¹. On the other hand, saxitoxin effects on the aquatic biota are relatively unexplored. To fill some of the existing gaps, this work aims at assessing the effects of a pulse acute exposure (24 h) to 30 µg L⁻¹ of saxitoxin on the model cladoceran *Daphnia magna*. A holistic approach anchored on the use of biochemical (antioxidant enzymes activity and lipid peroxidation), genotoxicity (alkaline comet assay), neurotoxicity (total cholinesterases activity), behavioral (swimming patterns), physiological (feeding rate and heart rate), and epigenetic (total 5-mC DNA methylation and total DNA methyltransferases activity) biomarkers was used to characterize saxitoxin toxic effects. Exposure resulted in decreased feeding rate, heart rate, total cholinesterases activity, swimming activity and catalase activity. Contrarily, other antioxidant enzymes, namely glutathione-S-transferases and selenium-dependent glutathione peroxidase had their activity increased, along with lipid peroxidation and DNA damage levels. Saxitoxin induced significant differences to epigenetic machinery enzymatic activity (DNA methyltransferases) that translated into significantly altered total DNA 5-mC levels. A putative adverse outcome pathway is proposed to help summarize the sub-lethal effects of saxitoxin on *Daphnia magna*.

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4.16.P-We405 *Marinogammarus marinus*, *Asparagopsis armata* and *Ostreopsis cf. siamensis*: the Good, the Bad and the Villain

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Global environmental changes are linked to the proliferation of harmful algal blooms (HABs) in coastal ecosystems, which can release high concentrations of toxic compounds. These toxins may harm resident biota and disrupt aquatic ecosystem dynamics. *Asparagopsis armata* is a non-indigenous red seaweed that forms blooms and produces a variety of toxic organohalogenes, while *Ostreopsis cf. siamensis* is a bloom forming unicellular benthic dinoflagellate with conflicting data available about its potential toxicity. This study assessed the ecotoxicological effects of these two algal species on *Marinogammarus marinus*, a representative coastal amphipod. Juvenile amphipods were exposed to increasing concentrations of macroalgal exudates (0–16%) and microalgal cell densities (0–14,566 cells·mL⁻¹) for 48 hours to evaluate survival. The LC50 values were determined to be 5.84% for macroalgal exudates and 452 cells·mL⁻¹ for microalgae. Subsequently, sublethal concentrations (LC10) of each stressor were tested individually and in combination, using a simple binary experimental design. Amphipods were exposed for 48 hours. After exposure, organisms were left to recover during 24h in a clean medium and were transferred to a medium with *Fucus* sp. Seaweed exudate (food stimulus), and their motility was tracked during 30s. Motility in amphipods was more affected by *O. cf. siamensis* LC10 than the equitoxic concentration of the exudate from *A. armata*. Amphipods were then tested for biochemical biomarkers indicative of oxidative (lipid peroxidation, LPO) and metabolic (electron transport system activity, ETS) stress, detoxification (glutathione S-transferase, GST), and neuroactivity (acetylcholinesterase, AChE).

Both algal species significantly induced lipid peroxidation and affected neuroactivity in the amphipods, which might be related to motility impairment. The dinoflagellate also inhibited ETS and GST activity, effects not observed with the macroalga and suggesting broader routes of toxicity by the dinoflagellate. Despite their distinct toxicity mechanisms, both species demonstrated significant negative impacts on *M. marinus*, highlighting potential risks to coastal trophic dynamics and ecosystem stability.

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4.16.P-We406 Hazard Characterization of the Marine Toxin Ovatoxin-a at the Skin Level

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Ovatoxin-a (OVTX-a) is the major palytoxin (PLTX) analogue identified in the benthic dinoflagellate *Ostreopsis cf. ovata* from the Mediterranean Sea. In this area, the risk for human health during *O. cf. ovata* blooms is mainly associated with inhalation of marine aerosol and/or skin contact with seawater. Despite the hazard posed by PLTX has been extensively characterized in the last decades, very few data are currently available for OVTX-a. Hence, this study was aimed at characterizing in vitro the hazard posed by skin exposure to OVTX-a, using spontaneously immortalized HaCaT keratinocytes as human epidermal cells.

The effects of OVTX-a (1x10⁻¹⁶–1x10⁻⁷ M) in HaCaT cells were compared to those of the reference toxin (PLTX) in terms of cell viability, cell necrosis, reactive oxygen species (ROS) production and mitochondrial depolarization. After 4 h exposure, OVTX-a induced a concentration-dependent cell viability reduction (EC50 = 8.3x10⁻⁹ M), with one order of magnitude lower potency than that of PLTX (EC50 = 3.7x10⁻¹⁰ M). Accordingly, OVTX-a induced a concentration-dependent increase of cell necrosis, with a potency lower than that of PLTX. Moreover, OVTX-a increased ROS production similarly to PLTX, but it caused a lower mitochondrial depolarization in keratinocytes with respect to the reference toxin. Even though with a different potency, OVTX-a and PLTX appear to induce these effects with a similar molecular mechanism as assessed co-exposing the cells to ouabain (OUA, 1.0x10⁻⁵ M), an

inhibitor of Na⁺/K⁺ ATPase and molecular target of PLTX, or diphenyliodonium chloride (DPI, 5.0x10⁻⁶), a non-specific inhibitor of flavoprotein-based enzymes known to be involved in PLTX-induced oxidative stress.

In addition, a particular focus was given to the inflammatory potential of OVTX-a, exposing HaCaT keratinocytes to its subcytotoxic concentrations up to 24 h, and analyzing the release of a panel of cytokines, chemokines (IL-1 α , IL-1 β , IL-6, IL-7, IL-8, IL-18, IL-33, TNF- α , IFN- γ , MCP-1, RANTES, GM-CSF, MIF, ECF-CCL11) and prostaglandin E2 (PGE2). In general, only the release of few cytokines (IL-6, IL-8, TNF- α) and PGE2 was significantly increased by OVTX-a exposure, albeit with a lower potency as compared to PLTX.

In conclusion, this study provides new evidences for the characterization of the hazard posed by OVTX-a towards skin keratinocytes.

4.16.P-We407 Detection of Potential Metabolites of Cylindrospermopsin in Rat Brain After Oral Exposure and Effects on Acetylcholinesterase Activity and on Oxidative Stress Biomarkers

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Cylindrospermopsin (CYN) is a cyanotoxin of increasing interest due to its toxicity and worldwide distribution. The liver is considered its main target organ, however, different studies show adverse effects also on other organs, including the brain. Despite this, few studies have focused on the impact of CYN on the nervous system and only two of them have determined the presence of CYN in brain of fish by enzyme-linked immunosorbent assay (ELISA). Moreover, to our knowledge, no studies have been performed in mammals with pure CYN. Therefore, this work is focused on the determination of CYN and its potential metabolites in the brain of male Wistar rats after oral exposure for 48 h to three different doses of pure CYN (7.5, 23.7 and 75 μ g/kg body weight) by ultra-high performance liquid chromatography coupled to a tandem mass spectrometry system (UHPLC-MS/MS). In addition, in order to determine the toxic effects of CYN on this organ, several biomarkers of oxidative stress, such as superoxide dismutase (SOD) and catalase (CAT) enzyme activities and lipid peroxidation (LPO) and glutathione (GSH) levels were evaluated, as well as the enzymatic activity of acetylcholinesterase (AChE). Although CYN was not detected in brain, up to 14 potential CYN metabolites produced by different metabolic pathways were identified. These potential CYN metabolites could arise via several routes, including phase I reactions: hydration, oxidative deamination to alcohol or oxidative deamination to ketone or phase II reactions, such as acetylation, methylation or conjugation reactions with glutamine, cysteine or arginine among others. Furthermore, the results showed a significant increase in SOD (1.7-fold) and CAT (1.3-fold) activities after exposure to the highest dose tested and a dose-dependent increase in LPO levels. However, no changes in GSH levels were detected. Regarding AChE activity, a significant reduction was observed at all doses used. Therefore, the observed alterations could be induced by CYN metabolites detected. This is the first study to link alterations at brain level to the presence of CYN metabolites. These results highlight the need for further studies focused on the potential adverse effects of CYN and its metabolites on the mammalian nervous system and its possible role in neurodegenerative diseases.

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4.16.P-We408 In Vitro Assessment of the Immunomodulatory Effects of Natural Lichen Extracts on Human Lymphocytes

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Lichens are symbiotic organisms known for their distinctive secondary metabolism. The metabolites produced by lichens demonstrate a variety of pharmacological activities, including antibacterial, antioxidant, and antiproliferative effects. *Pseudevernia furfuracea* (L.) Zopf is a lichen present in different localities in Morocco whose extracts have been previously characterised, with atraric acid as the main component. The purpose of this work was to assess the impact of two lichen extracts (HE1, HE2) in

representative cells of the immune system, the Jurkat cell line (lymphocytes). The results showed that both extracts reduced cell viability after 24 hours of exposure. The mean effective concentrations 50 (EC50) were $55.20 \pm 7.06 \mu\text{g/mL}$ for HE1 and $56.86 \pm 4.05 \mu\text{g/mL}$ for HE2. In addition, cell death mechanisms were investigated by flow cytometry, being the apoptosis and late apoptosis the predominant processes after 24 and 48 hours of exposure, respectively. Moreover, the mRNA expression of various cytokines was studied by RT-qPCR. Among them, interleukin 2 (IL-2) and interferon-gamma (INF- γ) levels were up-regulated after 24 hours of exposure to both HE1 and HE2, while a down-regulation was observed in the tumor necrosis factor-alpha (TNF- α) under the same experimental conditions. After 48 hours of exposure, only up-regulation of IL-2 was observed. Overall, these findings suggest that both extracts could have immunomodulatory effects in vitro. Further research is needed to determine the mechanisms involved and the impact of HE1 and HE2 on the human immune system.

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4.16.P-We409 A MALDI-MSI-Based Approach to Characterize the Spatial Distribution of Cylindrospermopsin and Lipid Alterations in Rat Intestinal Tissue

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Anthropogenic activities and climate change are exacerbating the proliferation and distribution of cyanobacteria, which produce harmful cyanotoxins like cylindrospermopsin (CYN). This toxin poses significant risks to both ecosystems and human health. Ingestion of contaminated food and water is the primary route of CYN exposure, underscoring the importance of monitoring its absorption, distribution, and potential health effects. In that sense, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry imaging (MALDI-MSI) offers a powerful tool for visualizing the spatial distribution of a wide range of molecules. This study aimed to develop and optimize a novel MALDI-MSI method for CYN detection in rat intestinal tissue. To investigate the quantitative spatio-temporal distribution of the toxin, mid-intestinal samples were collected from rats exposed to 500 μg CYN/kg body weight and sacrificed at 0, 2, 4, 6, and 24 hours post-exposure. Cryosections of fresh intestine tissue (jejunum) were obtained at 10 μm , mounted in MALDI IntelliSlides and frozen until use. The slides were allowed to reach room temperature during a 30 min drying step using a vacuum pump, and the DHB matrix was applied over the entire surface of the slide by using a HTX TM-Sprayer. MALDI-MSI experiments were carried out in positive ionization mode, within a m/z range of 300-1350 and an ion mobility range from 0.8-1.68, using a timsTOF-Flex mass spectrometer. Additionally, the impact of CYN on the intestinal lipid profile was assessed. The results showed that the method developed was useful to detect and quantify CYN and its sodium and potassium adducts in rat intestine. Moreover, the method was validated for linearity, sensitivity, and precision using mimetic tissue sections spiked with various CYN concentrations (0.1-100 ppm), ensuring its suitability for visualizing CYN, and its sodium and potassium adducts distribution in the rat tissue. Regarding lipid profile, significant alterations in several lipid families were observed, suggesting an inflammatory response, increased oxidative stress, and progressive damage to cell membrane integrity. Further research is necessary to elucidate the distribution of CYN in other vital organs, such as the liver, kidney, and stomach.

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4.16.P-We410 Assessing the Effects of Phycotoxin Mixtures on Marine Zooplankton: Insights from Copepod Responses at Different Life Stages

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The expansion of human activities into oceans and lakes has profoundly disrupted aquatic ecosystems. Over recent decades, HAB occurrences have increased due to mounting anthropogenic pressures. Harmful algal blooms (HABs) are a phenomenon occurring when certain primary producers, capable of generating toxic metabolites (phycotoxins) proliferate excessively. These phycotoxins can accumulate in fish and shellfish, subsequently moving up the food web and adversely affecting organisms at higher trophic levels, ultimately posing significant risks to human health. Phycotoxins are classified into five main groups based on their effects: paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP), diarrhetic shellfish poisoning (DSP), and azaspiracid poisoning (AZP). While the individual effects of these toxins are well-documented, the combined effects on the marine food chain remain less understood. Copepods are key primary consumers in marine ecosystems, acting as vital links to higher trophic levels, such as planktivorous fish. They also play an essential role in oceanic biogeochemical cycling, for example through carbon C and nitrogen export to deeper waters. This study, therefore, examines the effects of mixed phycotoxin exposure on two copepod species: the epibenthic copepod *Nitokra spinipes* and the planktonic copepod *Acartia clausi*. We investigated the impacts of two harmful algal species, *Protoceratium reticulatum* and *Alexandrium minimum*, using a full factorial design that included environmentally relevant concentrations of both living algae, as well as their extracts. After 48 hours of exposure to these mixtures, we evaluated the response of adult copepods for swimming speed, inactivity, and mortality using the ZebraBox device, while naupliar immobility was assessed under a light microscope. Our research aims to enhance understanding of the impacts and mechanisms of mixed HAB exposure on copepods, contributing to broader insights into potential risks to ecosystems and human health.

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4.16.P-We411 A Step Closer to Link LC-PUFA Fatty Acids and Toxin Biosynthesis in Toxic Dinoflagellates

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Long-chain polyunsaturated fatty acids (LC-PUFAs) are essential for many physiological processes in marine organisms. While these compounds are typically associated with health benefits in higher trophic levels, emerging evidence suggests that their presence in toxic harmful algae species (HABs) may be related to toxicity. While direct evidence is limited, PUFAs might serve as biochemical precursors or modulators in the biosynthetic pathways of certain algal toxins. In dinoflagellates it is believed that synthesis of some FAs and toxins share the same anaerobic Polyketide Synthase (PKS) pathway. This study explores the potential role of LC-PUFAs as proxies for toxin production in the benthic harmful dinoflagellates *Prorocentrum lima* and *Amphidinium carterae*. Both species are known for their production of bioactive secondary metabolites such as okadaic acid and amphidinols, respectively, potentially harmful to marine biota. Here, the toxicity of dinoflagellate species was firstly evaluated and validated through lethal effects (LC50) in marine microinvertebrates (artemia and amphipods). The levels of specific fatty acids were then correlated with the most common toxins in both dinoflagellate species, after culturing them at different temperatures (15°C, 19°C and 24°C), until both reached the end of their exponential growth phase. Results revealed strong positive correlations, mostly between EPA and DHA, and the quantity of toxins identified in both species.

Understanding these mechanisms is critical for predicting the ecological impacts of HABs, as disentangling the specific relation of particular fatty acids and other algal metabolites with overall toxins and their toxicity may become crucial in understanding and mitigating HABs effects on marine food webs.

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4.16.P-We412 A *Hyalella azteca* Transgenerational Biotest to Screen Reproductive and Embryonic Development Effects of MC-LR and Exudates from Cyanobacterial Toxic Strains

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Microcystis aeruginosa is known to its capacity to produce toxic secondary metabolites. These compounds pose a significant risk to water quality, human health, and wild life. Several studies pointed the effects of these compounds on invertebrates. However, the utilization of amphipods as a model organism remains limited. Based on a new two-week biotest, the aim of this study was to investigate the reproductive responses of *H. azteca* exposed to the cyanotoxin MC-LR (model substance) and MaE (complex mixture), and to evaluate their effects on the parental and F1 generations. Cyanobacteria was cultivated under controlled conditions until it reached a state of growth stabilization. The MaE was collected and assays were performed using a concentration of 100%. For the MC-LR toxin, tests were carried out at concentrations between 0.3 and 27 µg/L. Couples in the post-molt stage were exposed to controls, MC-LR and MaE solutions for six days. The survival of both sexes, progress of females in the molting stage, number of embryos present in females marsupium, and the stage of embryonic development were recorded. To assess the effects of parental exposure on fertility, the assays continued in uncontaminated water for a total of 13 days to measure the new production of F1 embryos correspondent to the first post-exposure laying event. At this point, the survival and body size of the females, number of embryos, embryonic development and malformation were evaluated. The results demonstrated that the survival of the organisms was not affected (at least 85% of survival in all samples). With regard to the reproductive response, MC-LR and MaE accelerated the molting process in females, although fertility was not affected. With respect to the embryonic development, no disorders were clearly observed when females were exposed to both samples. Consequently, it can thus be concluded that the MC-LR toxin has the potential to disrupt the molting dynamics of *H. azteca* females, a hypothesis that can be confirmed through the analysis of MaE responses since the strain in question is known to produce MC. The subsequent step will be to evaluate the effects observed in the F1 generation following parental exposure. The new findings of this endocrine disruption potential in the amphipods paves the way for future applications of *Hyalella* biotest for biomonitor the occurrence and the toxic potential cyanobacterial toxic strains in the aquatic environment and water resources.

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4.16.P-We413 Cyanobacteria Survival After Exposure to UVB Radiation

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In the context of climate change, shifts in global temperature and increased solar radiation are altering aquatic ecosystems, enhancing the frequency and intensity of harmful algal blooms (HABs).

Cyanobacteria, a dominant group in many freshwater and marine environments, are sensitive to changes in ultraviolet-B (UVB) radiation a factor exacerbated by ozone depletion and atmospheric alterations. UVB exposure triggers oxidative stress in cyanobacteria, potentially impacting their survival and ecological dynamics. This study investigates the survival rates and oxidative stress responses of *Nostoc* sp. PCC 7906 under controlled UVB exposure (1 min, 30 min, 1 h, and 3 h), aiming to better understand how these organisms may adapt or become vulnerable in a changing climate. A comprehensive array of biochemical biomarkers, namely the activity of the main antioxidant enzymes and thiobarbituric acid reactive substances (TBARS) levels as an indicator of oxidative stress onset were evaluated immediately after the exposure and then again after a recovery period of 38 days of culturing, to observe if antioxidative defense mechanisms could help exposed organisms recover from the damage caused by UVB.

Results showed that even the longest exposure (3 h) was not enough to kill the cells. Although their growth was impaired at the beginning, after ca. 1 week recovery, the cells started to grow and surpassed all the other treatments. Regarding the antioxidative metabolism, catalase was the only enzyme with its activity significantly increased in response to shorter exposures (1 and 30 min), while after 1 h and 3 h of exposure to UVB, the other antioxidative enzymes such as Glutathione reductase and Glutathione S-Transferase also had their activity significantly increased. TBARS levels were also significantly higher in cells exposed for longer (1 h and 3 h). These results seem to suggest that, despite the activation of the antioxidative defenses, UVB exposure caused oxidative stress. However, oxidative damage was higher immediately after the exposure, and diminished with time, suggesting that specific repair mechanisms were activated by the cells.

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4.16.P-We414 Developing High Throughput Screening Approaches to Characterize Cyanobacterial Sensitivity to Herbicides

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Development of novel herbicides to address cyanobacteria blooms often utilize flask-based experiments to identify sensitivity thresholds. Different cyanobacteria are sensitive to different herbicides, and it is challenging to adequately define effect concentration (EC) thresholds using current methodologies. High throughput approaches using microplates can streamline testing. However, there are nuances in growing conditions that can influence the results. The research had several aims: 1) investigate differences in growth rates and quality of cyanobacteria in flasks and microplates, 2) assess whether there were differences in interpretation of herbicide toxicity between the two culturing methods, and 3) identify the best method for investigating herbicide toxicity towards cyanobacteria. The study utilized *Microcystis aeruginosa* (UTEX 3037) due to their capacity to form uniform test suspension and relatively easy growing conditions. *Microcystis aeruginosa* were grown in flasks and microplates and cell viability and abundance was counted through flow cytometry for two weeks. Throughout the experiment, cell viability was maintained at greater than 99% in both the flask and microplate. Density of viable cells initially lagged in the microplate however, density increased rapidly on day 3 and exceeded densities of *M. aeruginosa* grown in the flasks on days 7, 11, and 14 demonstrating that growing conditions in the microplate did not influence *M. aeruginosa*. The previously described method was used to identify if dose response thresholds for copper were influenced by culturing method. *Microcystis aeruginosa* were exposed to copper using six treatments ranging from 5 to 160 µg/L. Chlorophyll and phycocyanin were measured through fluorescence using a microplate reader and cell viability and density were characterized through flow cytometry. Effect concentration thresholds demonstrated consistency between microplates, highlighting inter-plate reliability, and comparability to the flask cultures. Results demonstrated that high throughput screening approaches can be designed to investigate effects of herbicides on *M. aeruginosa* and that culturing conditions in the microplate did not influence the interpretation of copper ecotoxicity. Follow-up research is needed to validate the high throughput approach for other cyanobacteria. Research investigating the capacity of microplates to characterize the ecotoxicity of hydrophobic chemicals is also needed to verify the method developed.

Track 5. Life Cycle Assessment and Footprinting

5.01 Pushing the Limits: Incorporating Absolute Limits in Life Cycle Assessment

5.01.T-01 Sustainability Challenges of Global Hydrogen Supply in the Context of Planetary Boundaries

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As global decarbonization efforts accelerate, hydrogen is increasingly recognized as a crucial energy carrier for the transition to a low-carbon future. However, the environmental assessment of hydrogen supply chains, including both production and transportation, within the framework of planetary boundaries (PB), remains insufficiently explored on a global scale.

This study addresses this gap by evaluating the environmental impacts of 800 potential global hydrogen supply chains, obtained by combining 32 production methods and 25 transportation options. The former include steam reforming, water electrolysis with bioenergy and carbon capture and storage (WE-BECCS), and aluminium combustion, among others. Transportation methods cover options like compressed hydrogen, liquid hydrogen, and liquid organic hydrogen carriers, all using pipeline, trucks or ships. Each alternative is evaluated in six regions before results are aggregated at the global level, thus capturing the influence of regional factors. We evaluate the global impacts of these hydrogen supply chains using the Planetary Boundary-Life Cycle Impact Assessment (PB-LCIA) method, which allows us to express life cycle inventories as impacts caused on ten control variables for the PBs.

Findings reveal that hydrogen is currently responsible for 15% of global impacts on CO₂-based control variables, using already 40% of the safe operating space (SOS) for all anthropogenic activities. Among the 800 hydrogen supply chains assessed, only 205 reduce PB transgressions compared to BAU. The most sustainable option is WE-BECCS with *Miscanthus*, which keeps Energy imbalance and CO₂ concentration PBs within the SOS, but pressures biodiversity due to increased land use. Aluminium

combustion outperforms WE with PV or wind thanks to avoided treatment of aluminium scrap. Interestingly, WE powered with current electricity mixes does not improve the BAU scenario. Compressed hydrogen by pipeline emerges as the best option for transportation, although this depends on the distance and the region. In China, combining this option with WE-wind losses to BAU from 1000 km upwards, while this breakeven distance is 2500 km in Europe. Hence, hydrogen policies must not only focus on production but also address the environmental impacts of transportation, as they could offset production gains.

This study highlights that the best options to decarbonize hydrogen supply must align with transitions in other sectors, which vary by region.

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5.01.T-02 Current Aviation Roadmaps are not within Planetary Boundaries

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Background and purpose: The aviation sector is a significant contributor to global warming, accounting for almost 4% of effective radiative forcing. In order to assess its climate footprint over time, the sector and its various stakeholders have developed numerous prospective scenarios incorporating different assumptions about traffic growth, fleet renewal, introduction of new technologies, and the use of alternative fuels. However, the various solutions proposed are only assessed through the prism of climate.

By reducing the scale from global to sectoral, the Planetary Boundaries (PB) framework can be used to perform an Absolute Environmental Sustainability Assessment (AESAs) of contrasted prospective scenarios for the aviation sector.

Based on recently developed tools and methodologies, this study brings methodological and conceptual advancements to enable dynamic and prospective AESAs and present applications to aviation prospective scenarios.

Methods: This study uses and develops the PB-LCIA methodology, which links LCA to the PB framework. By using the premise tool and developing a dynamic approach to impact assessment, it develops a new methodology for prospective and dynamic AESA.

It also deploys and develops the "Fulfilment of Human Needs" approach of Heide et al. which is based on the notion of sufficientarianism to share PB budget between different human activities.

Results: Historical aviation already exceeds its PB budget for climate change, biosphere integrity, and nitrogen cycle. Scenarios using alternative fuels have a lower climate impact than the 100% fossil fuel scenario, but generate new potential environmental problems, such as the disruption of the phosphorus cycle. Among the 217 scenarios studied, none can be classified as absolutely sustainable.

Conclusions: By using new tools and developing an appropriate methodology, our work highlights the potential and the limits of alternative fuels to reduce the environmental impacts of aviation. It also shows the absolute environmental unsustainability of the aviation sector. Furthermore, the development of this methodology opens the possibility of conducting prospective and dynamic AESAs for other sectors of activity, enabling the anticipation of sustainability issues as well as the displacement of environmental problems in a context of decarbonization of activities.

5.01.T-03 Is the UK Consumption within Planetary Boundaries? A Supply Chain Perspective

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The Planetary Boundaries (PBs) framework has been operationalized at national and regional scales to evaluate absolute environmental sustainability. However, studies tracing the pressure leading to PBs transgressions in an integrated and comprehensive perspective remain limited. This study presents an absolute environmental sustainability assessment (AESAs) of UK consumption that focuses on tracing impacts from domestic production and foreign production (i.e. imported goods and services). The UK's consumptive environmental footprints are derived via an environmental extended multi-regional input-output analysis (EEMRIOA) with the Exiobase database, and then translated into PBs-related metrics through a refined regional-specific characterisation factors (CFs) model. Sectoral contribution analysis revealed that the agricultural and transport sectors are the two largest contributors to most PBs transgressions. Time series analysis demonstrates that while the UK's climate change-related PBs pressure

has declined over time, the country still exhibits significant transgressions due to outsourcing impacts to climate-intensive imported products. Other agriculture-related PBs, such as freshwater use, biogeochemical flows, and land system change present substantial increases over time and may trigger regional PBs transgressions in other supplier countries. The findings suggest that net importing countries like the UK should not only look at adjusting their consumption patterns, but also potentially better scrutinize their international trading partners. The study also highlights the importance of the supply chain perspective for comprehensive national/regional-level AESA.

5.01.T-04 Aligning Science-Based Targets with Consumer Needs: A New Method for Setting Climate Goals for Products

Teddy Serrano, Solene Boivent, Anders Bjorn and Michael Hauschild, *Technical University of Denmark, Denmark*

Operating within the Earth's carrying capacities requires the allocation of environmental budgets and targets at more granular levels, such as at the company level. Current frameworks, like the Sectoral Decarbonization Approach, developed by the Science-Based Targets initiative, focus on production intensity targets, which overlooks the role of products in fulfilling societal needs. This study explores the development of a new methodology that connects the allowed climate impact of companies products to their ability to fulfill the needs of final consumers.

The framework begins with a global emissions scenario aligned with the Paris Agreement to 2050, allocating budgets at country level based on an equal per capita approach. National budgets from 2022 to 2050 are distributed across functions, with 2022 reflecting current patterns and 2050 aligning with basic needs, defined by emissions from the lowest-impact households. In parallel, activity levels to 2050 are projected under two scenarios: business-as-usual (BAU) and sufficiency, setting upper and lower boundaries. Dynamic intensity targets, expressed in kg CO₂-eq per functional unit, are derived from the ratio of allocated budgets to activity levels.

This methodology is applied to short-distance mobility in Denmark and Spain as a proof-of-concept. The intensity targets are compared to the impact of current available technologies, calculated using LCA. This approach identifies when a product may become unsustainable in absolute terms or determines the rate of improvement needed for it to stay within its allocated environmental limits. Results highlight that sufficiency measures, such as limiting consumption to decent living standards, increase environmental space per activity unit, easing the pressure on technologies to meet stringent thresholds.

While this framework is designed for business-to-consumer companies, it can be expanded to business-to-business ones by linking their activities to the end products they support. Future research can also refine the methodology using prospective life cycle assessments to monitor technological alignment with dynamic targets.

5.01.P-Mo402 Inclusion of Albedo-Induced Climate Impacts of Land Use in Planetary Boundaries-based Life Cycle Assessment

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Land-use changes affect the climate not only through greenhouse gas (GHG) emissions but also through changes to biogeophysical properties such as surface albedo the fraction of solar radiation reflected by the earth's surface. Changes to the surface albedo alter the earth's energy balance causing radiative forcings that can rival the climate impacts of GHG emissions. The planetary boundaries framework highlights surface albedo changes as a key anthropogenic driver of climate change, alongside GHG and aerosol emissions. Despite this, the integration of albedo-induced impacts into life cycle assessment (LCA) and absolute sustainability assessment such as planetary boundaries-based LCA (PB-LCA) remains limited. This study presents a spatially and temporally differentiated method to quantify the climate impacts of surface albedo changes. We developed localized monthly characterization factors for various land use types using potential natural vegetation as a baseline to represent preindustrial conditions. We determined surface albedo changes by combining satellite data with a novel algorithm to predict the albedo of multiple land cover types for the same location. Methods to calculate the impacts both in radiative forcings (consistent with the planetary boundaries control variable) and kg CO₂e (consistent with more traditional LCA impact assessment methods) are presented. The results show that the albedo-induced climate impacts often greatly exceed the planetary boundary of +1 W/m² at local scales. However, to adequately compare

these localized limits to this global boundary, it likely needs to be downscaled to local levels. There is also pronounced intra-annual variability in the impacts, particularly in more northern regions, meaning that the boundary could be transgressed in only some months of the year, which still has the potential to trigger non-reversible tipping point reactions. However, for several land use types investigated here, the impacts are largely negative, reducing the climate burden. The albedo-induced climate impacts can indeed contribute to the potential transgression of the climate change boundary, especially when considering intra-annual variations. This demonstrates the importance of including these impacts into absolute sustainability assessments such as PB-CA. Impacts are also calculated in units of kg CO₂e, which is a less relevant metric for PB-LCA but is included to facilitate use with more traditional impact assessment methods.

Disclaimer/Disclosure: The authors would like to acknowledge the financial support of the industrial partners of the Consortium on Life Cycle Assessment and Sustainable Transition (a research unit of the CIRAIG). The authors remain solely responsible for the content of this study.

5.01.P-Mo409 A Boundary for Global Abiotic Resource Use: Constraints from Limited Regional Water Availability

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Abiotic resource production is essential for global economic development and the transition to low-carbon technologies but comes with significant environmental consequences, particularly through the use of regionally available water. This study examines the global production capacity of 32 abiotic resources, considering regional water availability as a key constraint. Our analysis reveals that current abiotic resource production exceeds water availability, with copper production in 2010 surpassing local water capacity by 37%. Relocating production to areas with lower water stress could alleviate these exceedances; however, economic factors often limit the feasibility of such shifts. Additionally, projected future demand for abiotic resources is expected to increase water consumption significantly. Incorporating water resource constraints into production strategies is crucial to ensuring sustainable resource management.

5.01.P-Mo410 Using Absolute Limits at Regional Level: Is Flanders Living Within its Fair Share of the Planetary Boundaries?

Karolien Peeters, An Vercalsteren and Maarten Christis, Flemish Institute for Technological Research (VITO), Belgium

Flanders, a region in Belgium, faces numerous challenges in maintaining a high-quality living environment. The Planetary Boundary (PB) framework is a widely recognised tool for assessing the stability of Earth System (ES) processes. This study explores the applicability of the PB framework in Flanders policy context, focusing on its potential to monitor the region's contributions to global and local environmental impacts. The research question is: Is Flanders living within its fair share of the planetary boundaries?

Applying the PBs concept at a sub-global scale requires translating globally defined limits to a regional context, a process that involves normative choices. Downscaling of the PBs to delineate the safe operating space for Flanders was done by applying the allocation principles Equality, Needs, Right to development, Sovereignty, Capability as proposed by EEA/FOEN. Each PB is assessed through control variables, yet these often differ from the indicators currently used by Flanders. The study involved the identification of the most appropriate control variables or proxy indicators for Flanders across all PBs.

The Flemish environmentally extended multi-region input-output (EE MR-IO) model was used to calculate footprint indicators for Flanders, where not yet available. By comparing these footprints with the safe operating space for Flanders, the study determined whether Flanders exceeds its fair share of the PBs. This analysis was done for those PBs for which regional data were available or could be easily calculated, being climate change, biosphere integrity, land system change, biogeochemical flows and freshwater use.

The study reveals that Flanders exceeds its safe share for four out of five ES processes, with freshwater use being the only exception. However, further research is necessary to define the best suitable proxy indicators to monitor PBs in Flanders, especially to address specific regional environmental issues. The study incorporated elements of Life Cycle Assessment (LCA) to define the boundaries for climate change

and biosphere integrity. However, the overall contribution of LCA remained limited. Conversely, useful insights were gained for applying absolute sustainability assessment within LCA. EE MR-IO models demonstrated potential for calculating sectoral contributions to ES process, providing a basis for setting sector-specific targets.

Disclaimer/Disclosure: Study commissioned by Departement Omgeving, Afdeling Vlaams Planbureau voor Omgeving.

5.01.P Pushing the Limits: Incorporating Absolute Limits in Life Cycle Assessment

5.01.P-Mo402 Inclusion of Albedo-Induced Climate Impacts of Land Use in Planetary Boundaries-based Life Cycle Assessment

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Land-use changes affect the climate not only through greenhouse gas (GHG) emissions but also through changes to biogeophysical properties such as surface albedo the fraction of solar radiation reflected by the earth's surface. Changes to the surface albedo alter the earth's energy balance causing radiative forcings that can rival the climate impacts of GHG emissions. The planetary boundaries framework highlights surface albedo changes as a key anthropogenic driver of climate change, alongside GHG and aerosol emissions. Despite this, the integration of albedo-induced impacts into life cycle assessment (LCA) and absolute sustainability assessment such as planetary boundaries-based LCA (PB-LCA) remains limited. This study presents a spatially and temporally differentiated method to quantify the climate impacts of surface albedo changes. We developed localized monthly characterization factors for various land use types using potential natural vegetation as a baseline to represent preindustrial conditions. We determined surface albedo changes by combining satellite data with a novel algorithm to predict the albedo of multiple land cover types for the same location. Methods to calculate the impacts both in radiative forcings (consistent with the planetary boundaries control variable) and kg CO₂e (consistent with more traditional LCA impact assessment methods) are presented. The results show that the albedo-induced climate impacts often greatly exceed the planetary boundary of +1 W/m² at local scales. However, to adequately compare these localized limits to this global boundary, it likely needs to be downscaled to local levels. There is also pronounced intra-annual variability in the impacts, particularly in more northern regions, meaning that the boundary could be transgressed in only some months of the year, which still has the potential to trigger non-reversible tipping point reactions. However, for several land use types investigated here, the impacts are largely negative, reducing the climate burden. The albedo-induced climate impacts can indeed contribute to the potential transgression of the climate change boundary, especially when considering intra-annual variations. This demonstrates the importance of including these impacts into absolute sustainability assessments such as PB-LCA. Impacts are also calculated in units of kg CO₂e, which is a less relevant metric for PB-LCA but is included to facilitate use with more traditional impact assessment methods.

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5.01.P-Mo403 Developing Characterization Factors and Carrying Capacities for Land Use Impacts on Ecosystem Functions Provision

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Anthropogenic land-use activities are the major cause of the Earth's biodiversity loss and therefore the exceedance of the planetary boundary for biosphere integrity, whose functional component is currently defined as the Human Appropriation of the biosphere's Net Primary Production (HANPP) smaller than 10% of the biosphere's net primary production. The focus on the potential loss and change of ecosystem functions from human activity like land use is complementary to more conventional indicators of loss in species richness and abundance.

In the community of life cycle impact assessment, a more complex set of Functional Diversity (FD) metrics, comprising Functional Richness (FRich), Functional Evenness (FEve), and Functional

Divergence (FDiv), have recently been proposed for measuring land use impacts on ecosystem function provision. It is currently unclear whether the HANPP indicator or the FD indicators are most sound for Absolute Environmental Sustainability Assessments (AESA). Regional boundaries (i.e., regional carrying capacities) for the FD indicators have not yet been quantified.

To fill this gap, we conduct an analysis to present 1) Sets of comparable Characterization Factors (CFs) for land-use impacts on ecosystem functioning taking HANPP and the FD metrics as indicators, at the same spatial resolutions within Europe and 2) regional boundaries estimation for each indicator.

Databases of plant traits, vegetation plots, patterns of land use and land cover, and HANPP are integrated to derive the CFs. To quantify the regional boundaries for the FD indicators, we rely on the identification of non-linearities in FD indicator values across different land use types for each natural vegetation zone. Tentative results demonstrate that the FRich of land is more significantly altered than FEve and FDiv under current land use patterns, and results of the FD indicators present patterns that do not fully overlap with those of the HANPP indicator. The final results are expected to reveal under what circumstances, if any, the HANPP indicator can serve as a reasonable proxy for the FD indicators in AESA.

Following additional research, CFs for ecosystem functioning, and their related planetary and regional boundaries, can be integrated with available AESA methods to support decision-making towards managing production and consumption activities within all planetary boundaries.

5.01.P-Mo404 Land-Use-Related Biodiversity Loss Embedded in Global Mining Supply Chains

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Global mining activities have undergone substantial expansion and intensification due to the escalating demand for minerals and metals, spurred by digitalization, infrastructure growth, and renewable energy transition. Such developments push Earth's system closer to the limit of the triple planetary crisis, particularly in biodiversity loss, including habitat destruction and landscape fragmentation. To mitigate mining-related biodiversity loss and align mining resource use with planetary boundaries, a spatially explicit assessment framework coupled with comprehensive supply chain analysis is urgently needed. Here, we first derived a land use and land cover map that categorizes mining land use separately.

Subsequently, absolute biodiversity loss from global mining was quantified by assessing local biodiversity intactness loss and global biodiversity importance at high spatial resolutions (10-arc-seconds and 30-arc-seconds). These biodiversity impacts were aggregated at the country level and allocated to specific mining commodities based on the monetary values of the respective extracted metal quantities in each country. Furthermore, the Resolved EXIOBASE dataset was used to link mining-related biodiversity loss from production to consumption perspectives. Our findings suggest that global mining leads to a 0.034 % global potentially disappeared fraction, accounting for nearly 70% of the planetary boundary budget for biodiversity loss. Biodiversity loss hotspots, including Indonesia, New Caledonia, Australia, Brazil, and Peru, account for 57% of the total impact. 82% of mining-related biodiversity impacts stem from extracting coal, precious metals, nickel, iron, and copper. Due to international trade, 77% of the mining-related biodiversity footprint is induced in countries other than final consumption. 58% of mining-related biodiversity footprint is driven by demand from China, Europe, Japan, and the USA, particularly in construction, services, machinery, and electronics. This analysis provides a holistic understanding of the absolute mining's biodiversity footprint and identifies levers to mitigate the adverse impacts. Further research is needed to standardize the mapping of mining land use, fill the data gap of mining production, and refine the assessment by increasing the taxonomic diversity and geographic representativeness, providing time series, and quantifying uncertainties arising from species ranges and taxonomic representativeness, among others.

5.01.P-Mo405 Just Allocation Principles for the Absolute Sustainability of European Battery Manufacturing

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The absolute sustainability of a sector based on the planetary boundary framework requires defining its share of safe operating space (SoSOS) which greatly influences the results. Current studies at country, regional or sector level apply ethical norms such as egalitarian, utilitarian, prioritarian, and inegalitarian principles - to calculate this SoSOS, often combining multiple approaches without justification. If the SoSOS definition is meant to be just, it is important to recognize varying definitions of justice.

Distributive justice includes not only egalitarian and prioritarian principles but also sufficientarian, principles of merit and limitarian principles. Furthermore, justice can be intragenerational but also intergenerational and interspecies. This study will calculate several SoSOS for the EU battery sector (consumption and production), while looking for justification to use SoSOS. Challenges arise from social acceptability and data availability.

To define a European SoSOS, various combinations of principles will be explored. The egalitarian perspective allocates equal SoSOS to all inhabitants of a region, advocating equal access to the ecological space. However, equity not only involves sharing resources, but also sharing rights, responsibilities, and risks. A SoSOS can be calculated for the current population. However, incorporating intergeneration criteria requires integrating future populations, which poses a challenge due to projections.

The utilitarian perspective considers sectoral utility, with economic added value, job creation, and physical output as potential bases. The sector's share in the European final consumption expenditure may also be used.

Under the prioritarian perspective, a right to development can be used based on the human development index. Applying historical emissions to a growing sector like batteries is complex. Priority could also be given to essential sectors such as health, agriculture, education, building, which exact list is to be defined. In an inequalitarian perspective, the grandfathering approach would be applied, basing SoSOS on current European and sector emissions.

It is recommended to use multiple sharing principles. This study will present a mix of established and innovative sharing principles. Although focused on the battery sector, the resulting SoSOS framework could apply to other sectors and countries, with global and local limits potentially treated differently.

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5.01.P-Mo406 Bringing Together Global and Regional Biophysical Limits of the German Bioeconomy and Corresponding Bioeconomy Scenarios Facilitating Absolute Environmental Assessment of the Sector – A Review

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The concept of bioeconomy oscillates between envisioning a potential high-tech future in which bio-based materials enable additional economic growth, or emphasizes biomass as a necessary means to replace fossil resources in a world characterized by reduced overall consumption. The given research aims to lay the groundwork to subject those visions to a reality check on the basis of Absolute Environmental Sustainability Assessment (AESAs).

In this review, the research aims to answer the question: How can global and regional impacts and biophysical limits of bioeconomy scenarios in Germany be quantified?

To answer this question, first, the work aims to review the research landscape for quantifiable biophysical limits, relevant as a sustainability benchmark to the German bioeconomy. While the review will lay an emphasis on the regional explicitness of biophysical limits at different scopes, it will also include examples of downscaled global limits. Second, the author aims to give an overview of established bioeconomy scenarios and modelling efforts. With this it is planned to review which of those fulfill the characteristics, needed for a global or regional AESA of the concept.

The research aims to build the basis for an AESA, as the result will establish an overview of available quantifications of limits and scenarios. Hereby, it takes a novel explicit regional and sectoral focus, advancing the research of AESA and bioeconomy monitoring.

5.01.P-Mo407 Operationalization of the Absolute Environmental Sustainability Assessment for the Safe and Sustainable by Design Framework: An Approach

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The Joint Research Centre's (JRC) Framework on Safe and Sustainable by Design (SSbD) chemicals and materials has relied, up to now, on traditional comparative Life Cycle Assessment (LCA) methods for evaluating environmental sustainability. However, the ever-growing global economy is pushing to the limit the earth systems, for which a new generation of absolute environmental sustainability assessment (AESAs) is being developed, by integrating the planetary boundaries (PBs) framework in the LCA. LCA-based AESAs has, until now, been applied primarily to established chemicals with known production processes and market shares. However, when conducting a sustainability assessment in the context of SSbD, it is essential to account for ongoing testing and for the iterative development process of new materials. The link between AESAs and the SSbD framework is explicit, but still lacks an operational perspective. Indeed, the actual global pressure of chemicals is still unknown, while, at the same time, the pace of production exceeds the capacity to test their environmental effects prior to the release on the market. Moreover, AESAs has not yet incorporated the boundary for Novel Entities (NEs) in any evaluation. Since SSbD chemicals and materials may qualify as novel entities both in a geological context and within the current market, operationalizing NEs assessment is crucial to integrate an absolute sustainability perspective in this context. For these reasons, within the EU SunRise and BioSusTex

projects, a multiple step approach is being developed, to adapt the absolute assessment to the stages of development of SSbD chemicals / materials. Chemicals pressure on the environment is being investigated for a specific set of materials through assessment in a lifecycle perspective. Safety testing, persistence analysis, and supply chain monitoring are the key aspects assessed to ensure respect for the boundary s threshold. On a bigger scale, the market sector for which the innovative chemical/material is designed is assessed against the planetary boundaries in the first development phases, when data availability is low. This is followed by an assessment of the product incorporating the innovation, aligning with a phase where more data can be collected. The results, in LCA fashion, would support the optimization processes during the design, allowing monitoring the transgression of the PBs and pointing out the hotspots of concern.

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5.01.P-Mo408 Translating Planetary Boundaries into Material-Level Life Cycle Assessments

Xue Sun¹, Stephan Pfister² and Roland Hirsch¹, (1)EMPA - Swiss Federal Laboratories for Materials Science and Technology, Switzerland, (2)ETH Zurich, Switzerland

As global environmental degradation accelerates, the Planetary Boundary (PB) framework provides a critical lens to evaluate social activities in relation to the ecological limits. At a material level, integrating the PB framework into Life Cycle Assessment (LCA) for an absolute assessment remains challenging, particularly for advanced materials highlighted in the EU CHIASMA project.

This contribution introduces a novel framework that translates global PBs into actionable, material-level LCA (PB-LCA) using a Multi-Layer Network Analysis and the Material Boundary Availability (MBA) method. Our network comprises three layers: key materials, life cycle stages, and nine PB dimensions. Analyzing network links, centrality, and edge weights identifies materials and life cycle stages that significantly influence the various PBs. By further evaluating each material s contributions to specific PB dimensions and defining maximum sustainable consumption levels across material consumption sectors, lifecycles, and use patterns, the MBA method quantitatively allocates safe operating space for materials within PBs. For instance, we allocate a portion of global CO₂ boundaries to a specific material sector based on its share of production, calculate its environmental impacts, and incrementally scale up until a boundary is violated at a chosen probability, yielding the maximum allowable volume for that sector. We provide practical insights to pinpoint materials most at risk of exceeding PBs and highlight them with visualization tools like heatmaps, enhancing data specificity and informing regulatory and industry practices for targeted interventions. Scenario analysis assesses the framework s robustness under these interventions to different degrees, addressing future uncertainties in technological developments, policy shifts, and circular economy practices. By combining the grandfathering allocation based on established shares and a sufficiency allocation rooted in basic needs fulfillment, the developed framework helps identifying a pathway from historical stability to a transitional, fair and sustainable future within Earth s ecological limits.

Finally, we present ideas for integrating PB-LCA into the Safe and Sustainable by Design (SSbD) framework. By developing material-level sustainability standards in future steps, our work would contribute valuable insights for globally guidelines and enhances datasets with key case studies from the CHIASMA framework.

5.01.P-Mo409 A Boundary for Global Abiotic Resource Use: Constraints from Limited Regional Water Availability

Kamrul Islam¹, Keitaro Maeno¹, Ryosuke Yokoi¹, Damien Giurco², Shigemi Kagawa³, Shinsuke Murakami⁴ and Masaharu Motoshita¹, (1)National Institute of Advanced Industrial Science and Technology, Japan, (2)University of Technology Sydney, Australia, (3)Kyushu University, Japan, (4)The University of Tokyo, Japan

Abiotic resource production is essential for global economic development and the transition to low-carbon technologies but comes with significant environmental consequences, particularly through the use of regionally available water. This study examines the global production capacity of 32 abiotic resources, considering regional water availability as a key constraint. Our analysis reveals that current abiotic resource production exceeds water availability, with copper production in 2010 surpassing local water capacity by 37%. Relocating production to areas with lower water stress could alleviate these exceedances; however, economic factors often limit the feasibility of such shifts. Additionally, projected future demand for abiotic resources is expected to increase water consumption significantly. Incorporating water resource constraints into production strategies is crucial to ensuring sustainable resource management.

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Karolien Peeters, An Vercalsteren and Maarten Christis, *Flemish Institute for Technological Research (VITO), Belgium*

Flanders, a region in Belgium, faces numerous challenges in maintaining a high-quality living environment. The Planetary Boundary (PB) framework is a widely recognised tool for assessing the stability of Earth System (ES) processes. This study explores the applicability of the PB framework in Flanders policy context, focusing on its potential to monitor the region's contributions to global and local environmental impacts. The research question is: Is Flanders living within its fair share of the planetary boundaries?

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The study reveals that Flanders exceeds its safe share for four out of five ES processes, with freshwater use being the only exception. However, further research is necessary to define the best suitable proxy indicators to monitor PBs in Flanders, especially to address specific regional environmental issues. The study incorporated elements of Life Cycle Assessment (LCA) to define the boundaries for climate change and biosphere integrity. However, the overall contribution of LCA remained limited. Conversely, useful insights were gained for applying absolute sustainability assessment within LCA. EE MR-IO models demonstrated potential for calculating sectoral contributions to ES process, providing a basis for setting sector-specific targets.

Disclaimer/Disclosure: Study commissioned by Departement Omgeving, Afdeling Vlaams Planbureau voor Omgeving.

5.01.P-Mo411 Challenges in Aligning the European Chemical Industry with Planetary Boundaries

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The European chemical industry plays a critical role in the region's economy, producing essential compounds for numerous sectors. However, its environmental footprint is also substantial, with high energy consumption, significant greenhouse gas emissions, and the release of harmful chemicals. Previous research on the environmental performance of the sector is limited to specific processes, lacking a comprehensive sector-wide perspective. To address this gap, here, we evaluate the sector's environmental performance and compare it against the planetary boundaries (PB), which defines the ecological limits for human activities to operate safely. This allows us to ascertain if the sector is sustainable and, if not, identify key areas of transgression.

We model the sector based on the 19 highest-volume chemicals, accounting for 80% of the industry's energy consumption and 75% of its greenhouse gas emissions. This highlights their critical role in both production volume and environmental impact. Given that each of these chemicals can be manufactured through multiple processes, our analysis incorporates data from 32 processes across 23 datasets, sourced from the ecoinvent 3.5 database. To avoid double counting impacts, we explore the links between these 23 activities and adjust production volumes accordingly.

Our findings reveal that the European chemical industry significantly exceeds multiple PBs, particularly climate change, ocean acidification, and biosphere integrity. The industry's contribution to atmospheric CO₂ concentration and energy imbalance at the top of the atmosphere exceeds safe levels by 15 and 16 times, respectively, while impacts on ocean acidification are 6 times greater than acceptable. The biosphere integrity boundary, assessed here via functional diversity, is also slightly transgressed (3%). Five high-volume chemicals ammonia, polypropylene, high-density polyethylene, styrene, and benzene are responsible for 50% of the sector's overall environmental burden across all PBs.

We also explore various mitigation pathways, including the deployment of carbon capture and storage (CCS) technologies, the use of renewable energy, and green hydrogen. Our results indicate that CCS could enable the sector to meet all PBs concurrently, yet burden-shifting remains a concern. Hence, these technological solutions must be implemented in conjunction with broader systemic changes, including policy interventions and cross-sector collaboration.

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5.01.P-Mo412 Combining Life Cycle Assessment and Planetary Boundaries in the Textile Fiber Sector: Absolute Environmental Sustainability Assessment of Regenerated Cellulosic Fibers

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The textile fiber sector has been growing for decades through the growth of synthetic fibers driven by, for instance, fast-fashion and increased welfare. The growth has fueled major environmental impacts. Wood-based regenerated cellulosic fibers (RCFs) may help in reducing the environmental impacts, though they may not be perfect substitutes for the established fiber types.

Life Cycle Assessment (LCA) is a standardized method to evaluate the environmental impacts of products, and it has been used to assess the environmental impacts of numerous products, including textile fibers. One of the shortcomings of LCA is its relativity: an LCA analysis can provide insights on whether a product causes more environmental burden than another product, but it cannot answer whether a product itself can be considered sustainable in the face of environmental crises. The Planetary Boundary framework provides a risk analysis for nine areas of Earth systems and the point at which humans operate currently relative to these areas and their safe operating spaces. In recent years, Absolute Environmental Sustainability Assessment (AESA) has been receiving growing interest as it aims to overcome the shortcoming of LCA by combining LCA with the PB framework to evaluate if a product can be considered sustainable in absolute terms.

This study will implement the AESA approach of combining LCA and the PBs to evaluate the absolute sustainability of the most established RCFs. The objectives are i) to allocate PBs for the RCFs with alternative approaches, ii) to calculate the life cycle impacts of the most common RCFs, and iii) to compare the LCA results to the defined PBs to examine the overall sustainability of the RCFs. One of the key challenges is to determine the planetary boundaries for RCFs, in which the choice of sharing principles is likely to influence the results. Therefore, sensitivity analysis will be a key part of the analysis.

This study will contribute to the existing AESA literature through a case study on textile fibers. It contributes further to the assessment of sustainability of wood uses, as wood-based textile markets are expected to grow in the future in the effort to reduce the textile sector's environmental impacts by RCFs substituting for the established fibers with greater environmental burden.

5.01.P-Mo413 Planetary Boundaries and Absolute Sustainability in LCA – Past, Present and Future

Efstathios Reppas Chrysovitsinos¹, Sarah Devecchi², Anna Lia Tromer Dragsdahl³, Stig Olsen³, Lisa Pizzol⁴, Elena Semenzin² and Tomas Vilhelm Rydberg¹, (1)Swedish Environmental Research Institute (IVL), Sweden, (2)Ca' Foscari University of Venice, Italy, (3)DTU, Denmark, (4)Green Decision, Italy Since the introduction of the Planetary boundaries concept in 2009 there has been an increasing interest in this concept which in its approach is based on a notion of finding an absolute threshold for sustainability on a global level. In line with this concept an adaptation was made to the LCA context by Bjorn et al, essentially redefining the planetary boundaries to LCA by calculating the threshold limits expressed in impact indicators as used in the LCA community.

With this study, the authors wanted to review the use of the planetary boundaries and absolute sustainability concepts in the LCA history as well as currently and provide a perspective on the anticipated use looking forward. The work carried out here has worked its way back through surveying both scientific and grey literature to find studies applying weighting of this third type and illustrate how they relate to the planetary boundaries, and some initial findings are presented.

The earliest method found to apply a distance to target approach for assessing LCA impacts was the eco-scarcity method. Later in the 1990-s, two approaches were developed independently in the Netherlands and the Nordic countries, the Eco-indicator 95 method and the ETlong method. Initial observations are that a boundary for absolute assessment is a very dependent on the criterium set for establishing the boundary. A further observation is that for many impact categories, although the boundaries are depending on the geographical location, a criterium can be applied by extrapolation or similar approach. But the

establishing of the criterium and thus the boundary will ultimately be done by a partly subjective decision. Work will continue towards a broader understanding of the prospects of absolute sustainability in LCA.

5.01.P-Mo414 A Step Beyond Life Cycle Thinking: Linking Supply Chain With Planetary Boundaries

Sara Toniolo and **Ivan Russo**, *University of Verona, Department of Management, Italy*

The sustainability of supply chain has emerged as a new stream for operations, which aims to actively create positive effects by designing supply chains that reduce the consumption of natural resources and generate social capital, also embracing the life cycle thinking. Going beyond this perspective means to acknowledge that mutual interactions exist along a supply chain and at different levels, interpreting it as a complex adaptative networks with interconnections with the natural system. This research proposes an analysis of the current level of interaction between supply chain and planetary boundaries, to identify areas for improvement and actions to be taken.

The intent of this research is to give an answer to the following research questions (RQ):

RQ1: How is the concept of planetary boundaries incorporated along a supply chain?

RQ2: How can the supply chain view and the life cycle perspective interact within the planetary boundaries?

The research is based on a systematic literature review and applies a life cycle thinking perspective, with the intent to consider the main environmental aspects associated with a supply chain.

A research by keywords was performed in ISI Web of Knowledge database in October 2024. The final combination of keywords was ("planetary boundaries OR planetary boundary) AND supply chain , leading to 33 articles. Then, based on a relevance analysis, 21 articles were finally selected. They were analyzed separately to identify how planetary boundaries can be incorporated along a supply chain, to address RQ1. Successively, they were crossed to identify how and if the life cycle perspective is applied or discussed, giving an answer to RQ2.

This research contributes to a comprehensive understanding of how to guide the work of supply chains within the planetary boundaries, showing how companies can operate also through the life cycle thinking. The research highlights the importance of measuring impact and performance outcomes to achieve sustainable operations and provides insights to assess and improve the sustainability level along a supply chain.

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5.02 Dimensions and Challenges of Life Cycle Assessment to Steer Innovation and Competitiveness of Safe and Sustainable Chemicals and Materials

5.02.P-Mo416 Estimating the Carbon Footprint of 130,000 Organic Chemicals with FineChem2

Dachuan Zhang¹, **Zhanyun Wang**², **Christopher Oberschelp**¹ and **Stefanie Hellweg**¹, *(1)ETH Zurich, Switzerland, (2)EMPA - Swiss Federal Laboratories for Materials Science and Technology, Switzerland*

Accurately understanding the product carbon footprints (PCFs) of chemicals is essential for promoting the sustainable transition of the chemical industry and its dependent sectors. In this study, we identified the limitations of traditional indicators such as molecular weight and market price for estimating PCFs, particularly for complex fine chemicals. To address these limitations, we developed an improved machine learning-based model with enhanced accuracy, expanded applicability, and robust uncertainty measures. Applying this approach, we analyzed PCF data for over 130,000 chemicals registered across 27 national inventories. Additionally, our analysis revealed that newly registered chemicals typically exhibit higher PCFs compared to existing ones, highlighting the urgent need for sustainable design guidelines.

Furthermore, we demonstrated that molecular frameworks significantly impact PCFs, with more ring structures often correlating with higher carbon emissions. We also found that chemical additives in plastics contribute substantially to PCFs, underscoring the risk of biased conclusions when these factors are overlooked. We anticipate this study will support more informed decision-making for chemical producers, consumers, and policymakers aiming to design and use more sustainable chemicals.

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5.02.P-Mo418 Integrating Risk Assessment and Life Cycle Assessment Methods in a Safe and Sustainable by Design Context

Neeraj Shandilya, Lia de Simon, Tom Ligthart and Wouter Fransman, TNO, Netherlands

It is crucial to assess the safety and sustainability of new chemicals or materials early in the design phase to ensure responsible development. The Safe and Sustainable by Design (SSbD) approach offers a comprehensive way to evaluate safety and sustainability from the outset, using human health risk assessment (HHRA) and life cycle assessment (LCA) to ensure long-term viability.

This work highlights the strengths and weaknesses of performing HHRA and LCA separately and demonstrates how to overcome these challenges in an SSbD context. Using perovskite-based solar cells (PSC) as a case study, we show the proof of concept. PSC offer high efficiency and low production costs but face issues with stability and material toxicity, which raise safety and sustainability concerns for their commercialization. The focus here is on demonstrating the integrated SSbD approach to support decision-making, rather than providing a detailed assessment of PSC safety and sustainability.

We demonstrated that without such an integrated approach, the comparative LCA does not cover the (absolute) human health risks associated with substances emission into the (work) environment. For the case of PSC, LCA showed human toxicity midpoint to be far less impactful than other midpoint categories (e.g. global warming, fine particulate matter formation) which might lead to its overlooking during decision making. On the other hand, integrating HHRA with LCA ensures a proactive risk aware approach that determines, in advance, the risks associated with human toxicity and calls for necessary chemical substitution during product design. Moreover, the integrated approach provides the necessary characterisation factors for an accurate LCA modelling in the relative toxicity impact categories.

Furthermore, the human toxicity midpoint within LCA only accounts for the general population exposure and excludes specific sub-groups like workers and consumers who could be more directly in contact with the toxic (volatile) substances. Through the integrated approach, we could account for a more detailed exposure assessment in such specific sub-groups.

Therefore, such an integrated HHRA-LCA approach can be beneficial for a holistic and yet meticulous assessment of a novel product for decision making in an SSbD context.

5.02.P-Mo423 Decision Support in Safe and Sustainable by Design: A Case Study in the Energy Storage Sector

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Stationary energy storage systems (ESS) are essential for stabilizing grids and ensuring reliable energy supply during the renewable energy transition. Redox flow batteries, as a promising ESS technology, offer scalability and long lifespans, but uncertainties about their safety and sustainability across the value chain necessitate further evaluation to address stakeholder concerns and guide ESS development. In this context, the safe and sustainable by design (SSbD) framework aims to minimize safety and sustainability risks in new materials through a holistic, life-cycle approach. However, uncertainties remain about its practical application to specific products and how to present results clearly to support decision-making at various levels. This study addresses these gaps by exploring the SSbD framework as a decision support tool at two levels: design and technology selection. An organic active element for redox flow batteries was chosen as the focus of the investigation. The assessments conducted (i.e., toxicity, environmental, social, economic, circularity and technical performance assessment) are based on the JRC SSbD framework, extended with use case-specific performance indicators. In each assessment step, several different indicators are evaluated (e.g., 16 impact categories for the life cycle assessment step), resulting in more than 30 indicators overall. Based on these results, a SSbD Matrix will be developed depicting the results in a heat map for the two decision-making levels (design and selection). To refine and validate the SSbD framework, a three-step approach for stakeholder involvement is planned: 1) A workshop with design engineers for the design support tool. 2) A workshop to discuss applicability with the SSbD community. 3) A stakeholder workshop for the selection support framework, with potential consumers (e.g., participants from the energy sector). The SSbD matrix, enabled a comprehensive comparison of electrolytes and batteries by integrating safety, sustainability, and technical performance indicators. It also serves as a stakeholder engagement tool, visualizing trade-offs and performance bottlenecks. By mapping lifecycle hotspots, the matrix provides targeted recommendations for design improvements, material substitution, supply chain management, and recycling strategies. Future research should focus on systematically weighting indicators and refining the matrix to enhance its precision and broader applicability.

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5.02.T-01 In Vitro & In Silico-Derived Effect Factors for USEtox: A Framework for Non-Animal Toxicity Characterization in Life Cycle Assessment

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The Horizon Europe project CHIASMA aligns with the European Commission's Safe and Sustainable by Design (SSbD) framework, aiming to integrate New Approach Methodologies (NAMs) into a robust system for human and environmental safety evaluations. CHIASMA focuses on incorporating in vitro and in silico data into the USEtox model to improve life cycle impact assessments (LCIA) by reducing reliance on animal testing.

Within CHIASMA, a stepwise approach advances Next Generation Safety Assessment (NGSA), refining USEtox for human and environmental toxicity characterization. This involves using physiologically-based kinetic (PBK) modeling and Quantitative Structure-Activity Relationships (QSAR) to estimate human-relevant doses, aligning with SSbD goals. It also incorporates the planetary boundary framework to evaluate absolute environmental sustainability.

The adaptation of USEtox to integrate in vitro and in silico-derived human toxicity effect factors (EFs) involves recalculating EFs based on non-animal data. CHIASMA uses QSAR and PBK models to estimate human-relevant doses from in vitro results. This process supports the operationalization of SSbD and aligns with LCIA goals.

Steps include:

Data Collection: Gather in vitro data on human toxicity endpoints and validate with in vivo data. QSAR models predict toxicity and benchmark doses for chemical analogues.

Benchmark Dose (BMD) Calculation: Adjust in vitro dosimetry to surface-area-based BMDs for standard comparisons with in vivo benchmarks.

Extrapolation Factors: Derive in vitro-to-in vivo extrapolation factors using the parallelogram approach and validate with QSAR/PBK models. Convert in vitro concentrations to human-relevant doses using PBK models.

EF Development: Integrate PBK-adjusted BMDs to calculate normalized human toxicity EFs consistent with USEtox standards.

Uncertainty Management: Use Monte Carlo simulations and dispersion factors to account for variability and enhance EF reliability.

The integration of these methods improves transparency and reliability. Adjustments are made for variability and uncertainty using Monte Carlo simulations, ensuring robust EF outputs. By operationalizing SSbD principles, CHIASMA provides a user-friendly framework for sustainable and safe chemical assessments.

This transformative approach demonstrates the feasibility of using non-animal data for regulatory applications while aligning safety evaluations with ecological and human health goals.

5.02.T-02 In-Silico Screening of New Chemicals for SSbD: Coupling Data-Driven Approaches and LCA

Gustavo Larrea-Gallegos and Antonino Marvuglia, Luxembourg Institute of Science and Technology (LIST), Luxembourg

With the introduction of the "Safe and Sustainable-by-Design" (SSbD) framework, the interest in practical applications of SSbD for new products has increased among policy, academic, and industrial players around the EU. During the development of new materials and products, the lack of information about impact pathways poses significant challenges. To ameliorate this problem, machine learning (ML) approaches can be used to screen the LCA impacts of new chemicals and materials.

In this study we applied an ML-based predictive model that can be used to estimate the impact of in-silico models of new chemicals. We selected chemicals included in the ecoinvent 3.10 database that could be associated with a Chemical Abstracts Service (CAS) number as training data. For them, we calculated their life cycle impact using the Environment Footprint (EF) method (19 impacts). The training features were physical-chemical descriptors generated using the chemicals' SMILE representation and the RDKit python toolkit. This finally yielded a data set consisting of 724 chemicals and ca. 200 descriptors. The model was trained following a conventional ML pipeline using the XGBoost algorithm (data curation, train/test split, hyperparameters search and cross-validation). We used the coefficient of determination

(R2) as performance metric, and we developed surrogate models to analyze feature importance and increase the explainability of the model.

The performance of our models (R2) ranged from 0.58 to 0.82 (Climate Change - biogenic and Climate Change, respectively) depending on the impact. For some impacts (e.g., Climate Change), our results matched and even outperformed other models reported in the literature. Our model can be used to provide fast and cost-effective estimations of LCA impacts of in-silico models of chemicals in the context of the SSbD framework. By using ML algorithms, we provided an end-to-end solution that can facilitate the screening of impacts when designing new chemicals. The novelty of our study relies on the use of a very recent life-cycle database and the consideration of the EF method for calculating impacts. Moreover, we also provided surrogate models to facilitate the understanding and reliability of our predictions. Finally, future steps will involve building a prospective screening LCA model by following the same ML pipeline, but incorporating prospective background inventories, and additional expert knowledge to describe the foreground system.

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5.02.T-03 Integrated SSbD Approach to Simulating the Sustainability of Lubricants: Challenges and Insights

Jonas Hoffmann and Andreas Ciroth, GreenDelta GmbH, Germany

The global drive to reduce carbon emissions highlights the importance of friction and wear management in reducing CO₂ emissions and global energy consumption. Lubricants are vital for the efficiency and durability of machinery, especially in low-carbon sectors like e-mobility and renewable energy. However, many conventional lubricants are environmentally harmful, necessitating the design of sustainable, non-toxic alternatives. The European Commission's Safe-and-Sustainable by Design (SSbD) framework aims to guide this process. Initial case studies show that SSbD assessment for marketed products can be time and cost-intensive.

The SiToLub project aims to address these challenges by simulating all SSbD dimensions - such as toxicology, biodegradability, and performance - into life cycle assessments (LCAs) of lubricants. This study compares a soybean-based bio-lubricant with a conventional petroleum-based lubricant using data from literature and a stoichiometric approach to model the bio-lubricant's life cycle. The cradle-to-gate impact assessment reveals that the bio-based lubricant has higher eutrophication, ecotoxicity, and toxicity impacts due to soybean cultivation, as expected. However, the fossil resource use and toxicity impacts were comparable, suggesting biases in comparing optimized petroleum-based processes with lab-scale bio-based ones.

This mismatch in Technology Readiness Levels (TRLs) poses a challenge in applying the SSbD framework effectively. The hierarchical approach used in low TRL development fails to capture the full sustainability of lubricants, particularly performance variations and end-of-life (EoL) scenarios. To improve this, the study incorporates EoL parameters like recyclability and recycling content into the life cycle model, reducing environmental impacts across relevant categories. Additionally, integrating QSAR methods for toxicology and biodegradability with reactive molecular dynamics simulations for performance offers a more comprehensive sustainability assessment.

Our findings highlight the limitations of the SSbD framework when applied to low TRL bio-based lubricants. A fully integrated SSbD approach, considering the entire life cycle and intrinsic properties like toxicity, performance, and biodegradability, is necessary for accurate sustainability assessments. Software-based solutions provide an efficient and cost-effective way to conduct these evaluations, addressing challenges in benchmarking new technologies.

5.02.T-04 Ecological Sustainability Assessment of Battery Energy Storage Systems: From Second-life to End-of-Life

Anna Spindlegger¹, Aleksander Jandric¹, Ricardo Gabbay De Souza², Stefanie Prenner³ and Florian Partl¹, (1)Institute of Waste Management and Circularity, BOKU University, Austria, (2) Technical University of Denmark, Denmark, (3)Brimatech Services GmbH, Austria

The increasing demand for electric vehicles and battery energy storage systems (BESS) is driving a surge

in the number of end-of-life lithium-ion batteries, necessitating effective end-of-life management strategies, such as repurposing and recycling. However, the implementation of these strategies faces challenges due to the diversity of battery designs, highlighting the importance of circular battery design principles. This study aims to assess the environmental impacts of different repurposing and recycling strategies for second-life BESS, providing insights that can guide improvements in the (re)design phase of lithium-ion batteries. A life cycle assessment was conducted on a second-life BESS, aligning with step 4 of the Safe and Sustainable by Design framework. Four repurposing cases were evaluated, focusing on the reuse of battery components in the second-life, and three cell recycling routes (pyrometallurgical, hydrometallurgical and advanced hydrometallurgical recycling). These results were then compared to a new BESS to determine environmental benefits or drawbacks of applying repurposed lithium-ion batteries. The results revealed a superior environmental performance of second-life BESS compared to new systems in four out of five analysed impact categories. The lowest impacts were identified when both the module casing and the battery management system are reused, while the highest impacts were observed when both components are replaced. The recycling phase demonstrated significant impact reductions, particularly in metal depletion and freshwater ecotoxicity. However, the reuse of the BMS significantly diminished these reductions for freshwater ecotoxicity, leading to environmental drawbacks of the repurposed system. Hydrometallurgical recycling methods achieved slightly higher mitigations due to the recovery of additional materials compared to pyrometallurgical recycling. This study underscores the potential of second-life BESS to reduce carbon emissions and decrease resource extraction. By evaluating various repurposing and recycling strategies, it highlights effective approaches to improve the environmental performance of second-life BESS. The findings emphasize the importance of considering end-of-life options in the battery design phase. Design for circularity can contribute to more efficient material recovery and improve the reusability of battery components, thereby improving the overall sustainability of battery energy storage systems.

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5.02.P Dimensions and Challenges of Life Cycle Assessment to Steer Innovation and Competitiveness of Safe and Sustainable Chemicals and Materials

5.02.P-Mo415 SaSo-HS tool: Leveraging Safety and Social Impact in the Safe and Sustainable by Design (SSbD) Framework

Antonio Nogueira, Beatriz Loureiro, Maria Pereira, Pablo Vicos and German Ferreira, HOLOSS, Portugal

The European Commission has launched the Safe and Sustainable by Design (SSbD) framework to promote innovation towards safer and more sustainable chemicals, materials, and products. The SSbD framework includes five key steps that integrate risk and sustainability assessments, with Life Cycle Assessment (LCA) covering environmental aspects. However, other sustainability dimensions such as economic and social are not yet fully developed or consistently applied. The social Life Cycle Assessment (s-LCA), which aims to operationalise social aspects, still faces significant challenges in terms of data granularity, availability, and applicability.

To address these limitations, this study introduces the SaSo-HS tool, developed by HOLOSS, to bridge the gap between safety and social assessments in the SSbD framework. The methodology includes conducting a comprehensive literature review to identify relevant areas that connect safety and social dimensions, designing a questionnaire to assess impacts in these areas, and developing a scoring system to transform qualitative data into semi-quantitative information. The SaSo-HS tool was iteratively refined to ensure it is comprehensive, user-friendly, and capable of providing holistic evaluations. It enables innovators to predict the impacts of their innovations across public health, urban health, vulnerable populations, toxicology regulations, social justice, and consumer trust.

The SaSo-HS tool provides a mechanism for innovators to assess the societal and safety impacts of new technologies in a structured and consistent manner. Future validation in relevant fields is essential to enhance the robustness of the tool. Expanding the survey scope to include new areas and additional

questions will also improve its comprehensiveness, helping to align innovations with the Sustainable Development Goals (SDGs) set by the United Nations. Ultimately, this integration of safety and social assessments seeks to support a more systemic, responsible, and sustainable development process, ensuring that new innovations benefit society while mitigating risks.

5.02.P-Mo416 Estimating the Carbon Footprint of 130,000 Organic Chemicals with FineChem2 *Dachuan Zhang¹, Zhanyun Wang², Christopher Oberschelp¹ and Stefanie Hellweg¹, (1)ETH Zurich, Switzerland, (2)EMPA - Swiss Federal Laboratories for Materials Science and Technology, Switzerland*

Accurately understanding the product carbon footprints (PCFs) of chemicals is essential for promoting the sustainable transition of the chemical industry and its dependent sectors. In this study, we identified the limitations of traditional indicators such as molecular weight and market price for estimating PCFs, particularly for complex fine chemicals. To address these limitations, we developed an improved machine learning-based model with enhanced accuracy, expanded applicability, and robust uncertainty measures. Applying this approach, we analyzed PCF data for over 130,000 chemicals registered across 27 national inventories. Additionally, our analysis revealed that newly registered chemicals typically exhibit higher PCFs compared to existing ones, highlighting the urgent need for sustainable design guidelines. Furthermore, we demonstrated that molecular frameworks significantly impact PCFs, with more ring structures often correlating with higher carbon emissions. We also found that chemical additives in plastics contribute substantially to PCFs, underscoring the risk of biased conclusions when these factors are overlooked. We anticipate this study will support more informed decision-making for chemical producers, consumers, and policymakers aiming to design and use more sustainable chemicals.

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5.02.P-Mo417 A Qualitative Social Life Cycle Assessment Approach for Early-Stage Safe and Sustainable by Design (SSbD) Projects

Daniela Groiss-Fuertner, Andreas Barth and Franziska Hesser, Wood K plus, Austria

Social Life Cycle Assessment (SLCA) is a key part of the Safe and Sustainable by Design (SSbD) framework, which evaluates social and socio-economic impacts. SLCA assesses factors like geopolitical conditions, industry sectors, cultural context, and individual perceptions. Early-stage material and value chain development (low Technology Readiness Levels, TRL) face challenges, such as uncertainties about stakeholders, production locations, and materials. In such stages, literature reviews are often used to understand potential social impacts, but specific knowledge on new value chains is limited. Thus, incorporating stakeholder perspectives is essential. The BIO-SUSHY case study, focused on developing innovative coatings for water- and oil-repellent applications aligned with the EU's chemical strategy, introduces a qualitative approach to address SLCA challenges in early stages. The goal is to identify social issues early in product development and provide guidance on improving social sustainability. SLCA follows the principles of environmental Life Cycle Assessment (LCA) with four steps: goal and scope definition, inventory, impact assessment, and interpretation. In the goal and scope phase, relevant impact categories are defined for specific stakeholders. SLCA complements environmental LCA by assessing potential social impacts across the product life cycle, identifying social hotspots along the value chain. Stakeholder involvement is highly recommended, and the BIO-SUSHY study introduces guidelines for qualitative expert interviews to facilitate this engagement at early TRL stages. Choosing the right interviewees is challenging due to limited knowledge about affected actors, but this can be addressed through in-depth value chain analysis. Integrating SLCA within the SSbD framework requires innovative methods to address knowledge gaps, often using a mix of approaches to prioritize social topics. Method selection depends on the TRL, complexity, and stakeholder knowledge. Engaging stakeholders provides specific perspectives and includes topics related to geographical, sectoral, or process-specific issues. Qualitative interviews offer in-depth insights into stakeholder experiences, preventing over-prioritization of commonly mentioned topics. The case study highlights issues like product quality, price, and technical requirements, with consumer topics also emerging, though often underrepresented in SLCA studies.

5.02.P-Mo418 Integrating Risk Assessment and Life Cycle Assessment Methods in a Safe and Sustainable by Design Context

Neeraj Shandilya, Lia de Simon, Tom Ligthart and Wouter Fransman, TNO, Netherlands

It is crucial to assess the safety and sustainability of new chemicals or materials early in the design phase to ensure responsible development. The Safe and Sustainable by Design (SSbD) approach offers a comprehensive way to evaluate safety and sustainability from the outset, using human health risk

assessment (HHRA) and life cycle assessment (LCA) to ensure long-term viability.

This work highlights the strengths and weaknesses of performing HHRA and LCA separately and demonstrates how to overcome these challenges in an SSbD context. Using perovskite-based solar cells (PSC) as a case study, we show the proof of concept. PSC offer high efficiency and low production costs but face issues with stability and material toxicity, which raise safety and sustainability concerns for their commercialization. The focus here is on demonstrating the integrated SSbD approach to support decision-making, rather than providing a detailed assessment of PSC safety and sustainability.

We demonstrated that without such an integrated approach, the comparative LCA does not cover the (absolute) human health risks associated with substances emission into the (work) environment. For the case of PSC, LCA showed human toxicity midpoint to be far less impactful than other midpoint categories (e.g. global warming, fine particulate matter formation) which might lead to its overlooking during decision making. On the other hand, integrating HHRA with LCA ensures a proactive risk aware approach that determines, in advance, the risks associated with human toxicity and calls for necessary chemical substitution during product design. Moreover, the integrated approach provides the necessary characterisation factors for an accurate LCA modelling in the relative toxicity impact categories. Furthermore, the human toxicity midpoint within LCA only accounts for the general population exposure and excludes specific sub-groups like workers and consumers who could be more directly in contact with the toxic (volatile) substances. Through the integrated approach, we could account for a more detailed exposure assessment in such specific sub-groups.

Therefore, such an integrated HHRA-LCA approach can be beneficial for a holistic and yet meticulous assessment of a novel product for decision making in an SSbD context.

5.02.P-Mo419 Integrating the ‘Safe and Sustainable-by-Design’ Principles with Life Cycle Assessment: A Practical List of Data Requirements and Approach for the Mineral Carbonation of Steel Slags Case Study

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Earlier this year in 2024, the European Commission Joint Research Center published a guidance document for the Safe and Sustainable-by-Design (SSbD) principles which provides methodological guidance to the application of the SSbD principles. The SSbD principles can provide a platform to integrate safety, economic, societal, and environmental design dimensions to design sustainable chemicals, materials, and processes. The incorporation of all these aspects could also be construed as an expansion to life cycle sustainability assessment (LCSA) with an integration of safety considerations. As this framework has just been published, there are still limited case studies, let alone actual approaches in real life to demonstrate the applicability of these SSbD principles. To this end, this study aims to compile and present a list of practical data requirements that would be necessary to perform a life cycle assessment (LCA) and to integrate LCA with the SSbD principles by means of a multi-criteria optimization analysis (MCOA). This study also outlines the process flow approach to envision how the required data can be used to calculate the environmental impacts and combine the end results with the SSbD principles, by using MCOA, with different assessments suggested for the case study involving the mineral carbonation of steel slags as a carbon capture, utilization and storage technology. Therefore, the outcome of this study can be used as a practical approach to generate data and continue with the assessments in order to satisfy all the requirements of the SSbD design principles. Future prospects would include the compilation of the data requirements for the economic and social assessments that can be combined to cover the whole range of the SSbD design principles. In addition to this, the practical approach is also planned to be applied in actual projects where future outcomes could help identify further areas of improvement in order to move a step closer towards a safe and sustainable manufacturing value chain.

5.02.P-Mo420 Monitoring and Evaluating Sustainable Public Procurement: A Multi-Level Methodology Integrating Environmentally Extended Input-Output Analysis and Life Cycle Assessment

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Procurement plays a key role in safety and sustainability within organizations. In 2019, the Dutch government spent 85 billion on products and services, accounting for 15% of total procurement in the Netherlands. This spending contributed to 18% of national consumption-based greenhouse gas emissions,

23% of resource use, and 9% of land use. These figures highlight the substantial environmental impact of public procurement, making it a key area for targeted sustainability policies.

Despite its potential, monitoring and evaluating Sustainable Public Procurement (SPP) remains a challenge. Traditional methods for monitoring and evaluation, which rely significantly on the involvement of busy procurement professionals, are often constrained by limited data and require significant time and resources. To address these challenges, we propose a multi-level methodology that combines Environmentally Extended Input-Output Analysis (EEIOA) with Life Cycle Assessment (LCA).

EEIOA provides a high-level analysis of the environmental impact per product group based on monetary flows, enabling the identification of the environmental hotspots. However, it lacks the granularity to guide specific interventions to procurers and suppliers alike. To bridge this gap, LCA is applied to the identified and prioritized hotspots, enabling detailed assessments of individual product groups. This lifecycle-based approach is relevant in the context of the Safe and Sustainable by Design framework, providing actionable insights to support decision-making in public procurement.

Beyond environmental factors, the approach also aims to integrate social dimensions, such as labor conditions and equity in supply chains, providing a holistic approach for evaluating SPP impacts. By combining EEIOA and LCA, this methodology has the potential to improve monitoring and evaluation processes, enabling policymakers to better track progress and refine strategies to achieve sustainability objectives.

While developed for public organizations in the Netherlands, this multi-level methodology could also be adapted to other contexts, offering a useful EEIOA and LCA approach to strengthen the role of procurement in driving sustainability globally.

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5.02.P-Mo421 Integrating Agent-Based Modeling and Life Cycle Assessment for Informed Decision-Making in the Energy Transition: A Case Study on Photovoltaics

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The energy transition involves shifting from traditional fossil fuels to renewable energy sources, a process that brings both environmental benefits and societal challenges. A key issue is how to guide this transition effectively, accounting for the complex interactions between energy systems, consumer behavior, and environmental outcomes. Conventional assessment methods often miss these dynamics, leading to incomplete or biased evaluations. To address this gap, we studied integrating Agent-Based Modeling (ABM) with Life Cycle Assessment (LCA) using a metamodel. This combination allows for real-time feedback between consumer decisions and environmental impacts, reducing computational intensity without sacrificing accuracy.

This approach was tested in a case study on photovoltaic (PV) panels, analyzing various scenarios to explore how end-of-life decisions, such as recycling or landfilling, affect environmental outcomes, including resource depletion and climate change. An existing LCA and ABM study on PV panels is adapted for this. The coupling of the two was realized by linking awareness of environmental impacts to consumer behavior towards circular pathways. We utilize concepts like thresholds of concern and thresholds of indifference to adjust consumer attitudes based on the calculated environmental impacts. This analysis revealed that consumer behavior can significantly influence environmental outcomes, particularly through changes in recycling and reuse rates, uncovering unexpected dynamics in sustainability transitions. This study showed that this is a viable approach to coupling an LCA and ABM in order to find potential tipping points in behavior. However, the outcome should be interpreted with care and more robust analysis should be developed to better analyze and understand the factors that are most influential in driving complex dynamics. This is a pioneering study illustrating the benefit and pitfalls of such complex analysis. Future work will expand the application of this integrated framework of LCA and ABM analysis to heat pumps and deepen the interpretation of emergent behaviors to better guide policymakers and stakeholders in the energy transition.

5.02.P-Mo422 Integrating BIM and LCA for Sustainable High-Speed Rail Infrastructure: A Framework for Early Design Stage Environmental Assessment

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The building and infrastructure sectors have a substantial impact on the global environment, underscoring the critical necessity for sustainable design practices. This study advocates for the integration of Building Information Modeling (BIM) and Life Cycle Assessment (LCA) to evaluate the environmental impacts of high-speed rail infrastructure projects from the initial design phases, by coupling Building Information Modeling's (BIM) data-filled 3D modeling with Life Cycle Assessment's (LCA) systematic evaluation of environmental impacts.

The objective is to enhance interoperability BIM and LCA tools in order to optimize design and reduce environmental impacts throughout the infrastructure's life cycle. We propose a methodological framework that involves thorough data collection from railway projects, covering design specifics, material specifications, and maintenance needs. An updated BIM-LCA integration framework has been developed using Ecoinvent 3.9.1 for comprehensive and accurate life cycle inventory data. Environmental indicators such as carbon footprint, energy consumption and waste generation are calculated using OpenLCA, allowing real-time feedback on performance. Sensitivity analyses have also been carried out to identify key design and operational factors influencing the environmental results.

Preliminary results from a high-speed rail bridge case study indicate that both the production and maintenance stages alone contribute between 93% and 99% of the impacts: water consumption, land use, freshwater ecotoxicity, human toxicity, marine ecotoxicity and mineral resource scarcity. A major portion of these impacts was attributed to the production of concrete elements such as piles, beams, and deck slabs. These initial findings highlight the importance of material selection in railway infrastructure projects to reduce their environmental impacts.

This study offers an insight on the potential of integrated BIM-LCA methodologies in promoting sustainable infrastructure design. This approach promotes informed decision-making by allowing for real-time assessment of environmental performance and enhancing data exchange within the BIM and LCA workflow.

5.02.P-Mo423 Decision Support in Safe and Sustainable by Design: A Case Study in the Energy Storage Sector

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Stationary energy storage systems (ESS) are essential for stabilizing grids and ensuring reliable energy supply during the renewable energy transition. Redox flow batteries, as a promising ESS technology, offer scalability and long lifespans, but uncertainties about their safety and sustainability across the value chain necessitate further evaluation to address stakeholder concerns and guide ESS development. In this context, the safe and sustainable by design (SSbD) framework aims to minimize safety and sustainability risks in new materials through a holistic, life-cycle approach. However, uncertainties remain about its practical application to specific products and how to present results clearly to support decision-making at various levels. This study addresses these gaps by exploring the SSbD framework as a decision support tool at two levels: design and technology selection. An organic active element for redox flow batteries was chosen as the focus of the investigation. The assessments conducted (i.e., toxicity, environmental, social, economic, circularity and technical performance assessment) are based on the JRC SSbD framework, extended with use case-specific performance indicators. In each assessment step, several different indicators are evaluated (e.g., 16 impact categories for the life cycle assessment step), resulting in more than 30 indicators overall. Based on these results, a SSbD Matrix will be developed depicting the results in a heat map for the two decision-making levels (design and selection). To refine and validate the SSbD framework, a three-step approach for stakeholder involvement is planned: 1) A workshop with design engineers for the design support tool. 2) A workshop to discuss applicability with the SSbD community. 3) A stakeholder workshop for the selection support framework, with potential consumers (e.g., participants from the energy sector). The SSbD matrix, enabled a comprehensive comparison of electrolytes and batteries by integrating safety, sustainability, and technical performance indicators. It also serves as a stakeholder engagement tool, visualizing trade-offs and performance bottlenecks. By mapping lifecycle hotspots, the matrix provides targeted recommendations for design improvements, material substitution, supply chain management, and recycling strategies. Future research should focus on systematically weighting indicators and refining the matrix to enhance its precision and broader applicability.

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5.02.P-Mo424 Parametric Life Cycle Assessment of Lithium Production: Prospective Model

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The European Union has banned the sale of thermal vehicles by 2035, which has increased the demand for electric vehicles. This has resulted in an increased demand for critical battery materials such as lithium. In this context, lithium refining and Lithium-ion battery production in Europe are subjects of global interest and sovereignty concerns. Therefore, there has been a rise of new integrated and non-integrated lithium projects worldwide. Subsequently, several actors are also interested in the environmental impact of battery components. Moreover, the European Union has created several regulations to quantify the environmental impact of battery components, including lithium hydroxide. Chemical (such as lithium) and process-based industries are liable for specific environmental legislation and sustainable practices, especially in the EU. In chemical engineering, the process selected is related to the feedstock impurity profile and the required purity of the final product. Consequently, environmental impacts will be highly variable depending on the feedstock type and processes and to evaluate these environmental impact, Life cycle assessment method (LCA) is used. There is a general lack of studies on the environmental impact of lithium with clear and transparent LCI data. This is the case for Lithium hydroxide production from hard rock and lithium carbonate to lithium hydroxide conversion. Moreover, there is also a lack of prospective modelisation of future lithium extraction and processing projects. This study will evaluate the environmental impact of lithium hydroxide production from spodumene and processed in China. This will be compared with different lithium hydroxide production LCA in the literature, with a focus on European Lithium production. This study will use data from academic and technical documents to collect foreground inventory data. It will use brightway 2 (and activity browser) to evaluate the environmental impact of lithium hydroxide. There will be also a sensitivity analysis by variating different foreground and background data to examine the potential prospective impacts.

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5.02.P-Mo425 Designing a Sustainable Future: Assessment of a Novel Niobium-Based Anode Material (XNO®) for Lithium-Ion Batteries Using the Safe and Sustainable by Design Framework *Ligia da Silva Lima¹, Jianshen Wu², Erasmo Cadena³, Alexander S. Groombridge² and Jo Dewulf³, (1)ARCHE Consulting, Belgium, (2)Echion Technologies LTD, United Kingdom, (3)Ghent University, Belgium*

Rechargeable batteries have become key for several sectors, from electronics to mobility, and play a pivotal role in the energy transition. Batteries not only support the decarbonization of mobility, but they also enable renewable energy storage, which is crucial for minimizing emissions across all industrial sectors. Demand for batteries and raw materials has increased exponentially over the past decades and is expected to continue rising. However, the supply of these materials is often linked to environmental burdens and social rights violations. Thus, responsibly managing the increasing supply of these technologies is critical to achieve a safe and sustainable energy transition. The Safe and Sustainable by Design (SSbD) framework aims to support the design of materials supply processes by implementing sustainable practices in two stages, namely Guiding (re)design principles (stage 1) and Safety and sustainability assessment (stage 2).

This study assesses a novel niobium-based anode material (XNO®) for lithium-ion batteries (LIBs) using the SSbD framework. The XNO® offers superior technical properties and has the potential to replace lithium titanate (LTO) and graphite anodes as it outperforms in several of the SSbD design principles. Some examples are material efficiency (less critical raw materials (CRMs) used), design for energy efficiency (20% less anode material is needed to deliver 1 kWh), use of renewables (renewable electricity in the production phase), design for end-of-life (key raw materials with expected good recovery properties) and consideration of the whole life cycle (from raw material acquisition to end-of-life). Regarding safety and sustainability, the XNO® shows positive results for human health and safety aspects in the production and use (low working conditions and social rights violations risks) and better environmental sustainability assessment, measured using life cycle assessment (e.g. global warming potential 51% lower than LTO and 48% lower than graphite). These results were obtained based on primary data for the XNO®, including battery design and manufacturing models, and literature data for LTO and graphite adapted into the scope of the study using the models.

The findings indicate that XNO® is a promising replacement for widely used anode materials such as graphite and LTO, enabling the substitution of CRMs in a safe and sustainable manner and exemplifying sustainable research and innovation within the SSbD framework.

Disclaimer/Disclosure: Part of the results of this work have been published (<https://www.sciencedirect.com/science/article/pii/S2214993723000891>) and part has not yet been published.

5.02.P-Mo426 LCA for Biowaste Turned into Value-Added Products

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As part of the transition towards a post-petroleum society, bio-based industries offer a unique opportunity to establish sustainable, innovative and competitive value chains. The European Commission adopted a new Circular Economy Action Plan in 2020 aimed at finding new ways to valorise high quality waste streams for reuse, remanufacturing and recycling, thus making the circular economy a reality. The CIRCULAR BIOCARBON project presents a first-of-a-kind flagship biorefinery designed to valorize the Organic Fraction of Municipal Solid Waste (OFMSW) and Sewage Sludge (SS) into high-value bio-based products, such as DLC coated products, biodegradable bags, graphene-based devices, compostable films, and biostimulants.

To demonstrate and validate the potential benefits of the project a triple-bottom sustainability impact assessment was conducted. The Environmental Life Cycle Assessment (LCA) was modeled using OpenLCA software based on the EF 3.1 impact assessment method.

Several challenges arose related to methodological decisions taken when modeling multi-output systems. As the biowaste entering the system model transitions from waste to resource, the LCA adopts a production perspective, focusing on the impacts directly associated with the production processes of each building block and bio-based materials. Therefore, several allocation criteria were studied throughout the assessment and a scenario evaluation as carried out.

Despite the promising outcomes, the study underscored the challenges posed by data availability, representativeness, and methodological subjectivity. These challenges complicate comparisons with fossil-based alternatives. This study emphasized the importance of transparent assumptions and the need for further sensitivity analysis, including a waste treatment approach with system expansion to better assess the role of waste treatment in a circular economy.

In conclusion, the variability in data availability, methodological choices, and allocation methods introduces uncertainty into the results, requiring careful interpretation. Hence, methodological choices have significant implications on the outcomes, underscoring the need for standardized approaches when assessing the environmental footprint of bio-based industries.

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5.02.P-Mo427 Applying the Safe-and-Sustainable-by-Design Framework: Life Cycle and Risk Assessment of a Microalgae-Based Product

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Europe is moving towards sustainability to address global environmental challenges such as climate change and environmental degradation. Initiatives such as the European Green Deal and the Chemicals Strategy for Sustainability aim to transform the European economy towards climate neutrality. In this context, the European Commission has developed the Safe-and-Sustainable-by-Design (SSbD) framework, aimed at improving the safety and sustainability of new and existing materials and chemicals throughout their life cycle. This approach integrates methods such as Risk Assessment (RA) and Life Cycle Assessment (LCA) to provide a holistic perspective encompassing health, safety, and environmental sustainability aspects.

This study, developed in the context of SCALE project funded by CBE JU, applies the SSbD framework to evaluate Brainphyt, a microalgae-based ingredient with applications in nutrition, animal feed and food supplements. The combined methodology integrates RA and LCA, focusing on phases 2 and 4 of the

SSbD framework. In phase 2, worker health and safety during production process is addressed through a risk analysis supported by the Social Hotspot Database. This analysis also includes impact categories such as human toxicity, both carcinogenic and non-carcinogenic, and freshwater ecotoxicity, as recommended by PEF methodology. In phase 4, environmental sustainability is assessed using LCA considering 12 environmental impact categories.

The results highlight that, in terms of health and safety, the chemical sector in France and the non-metallic minerals sector in Belgium are the largest contributors to the overall impact. In the categories of human toxicity and ecotoxicity, the use of the photobioreactor and the production of the culture medium account for more than 60% of the total impact, mainly due to the intensive use of chemicals at these stages. In the environmental assessment, the categories with the highest contributions, such as global warming potential, terrestrial ecotoxicity and land use, are also dominated by the culture medium and the photobioreactor. This study highlights the applicability of the SSbD approach to assess emerging products, providing a holistic perspective of their health and sustainability impacts. It underlines the need to optimise critical processes in Brainphyt's production to reduce its environmental impacts and improve safety along the production chain, moving towards a sustainable bioeconomy in the European Union.

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5.02.P-Mo428 Applying the Three Dimensions of Sustainability of the Safe and Sustainable by Design framework to Evaluate the Shift From Bisphenol-A-Based to Lignin-Based Epoxy Resins

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Epoxy resins are synthetic polymers with high-value adhesive and resistance properties used in several industries such as art, automotive, construction and electronics. However, a widely used chemical to produce epoxy resins is Bisphenol A (BPA), a chemical classified as 'Substance of Very High Concern' due to its endocrine disrupting properties. Therefore, lately, concerns regarding the epoxy resins maintenance, end of use and recyclability have been increasing since treating these materials provokes a substantial leak of BPA to the environment.

In this context, the LEGACY project a project funded by NextGenerationEU - aims to produce biobased epoxy resins (using lignin), assuring that the substitution will be superior from both a sustainability and a safety point of view. To substantiate this, the Safe and Sustainable by Design (SSbD) framework will be used, where applications of biobased epoxy coating will be fully assessed through all steps in the extended framework: hazard analysis, human health and safety assessment, environmental assessment and a social and economic assessment. As such, the project will implement all three dimensions of sustainability in the context of the SSbD framework. Combining these results will enable the identification of trade-offs. The newly developed BPA-free product will be tested in two use cases in collaboration with involved companies concerning the application of biobased epoxy resins in coatings. The two demonstrators are situated in the flooring and the sewer piping sectors.

Due to the low Technology Readiness Level (TRL), the environmental impacts will be assessed by means of a prospective Life Cycle Assessment (LCA). The results will be compared to those of the BPA-based resins as a benchmark. Using a scoring system based on the percentage improvements, the impacts will be rated and assigned a level.

The economic impact of the whole value chain of these case studies will also be calculated through a techno-economic analysis (TEA), based on a market study, process flow diagrams and mass and energy balances. Due to the low TRL, the focus will be on identifying the most important parameters that influence the economic feasibility.

Furthermore, the social dimension will be assessed following the UNEP guidelines for social life cycle assessment. The reference scale approach will be followed.

This project plan and approach will be elaborately explained in the poster presentation.

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5.02.P-Mo429 Safe and Sustainable by Design (SSbD) MAX Phases and MXenes: Environmental Impacts Comparison Through Life Cycle Assessment (LCA)

Valeria Acevedo García and Carlos Bernárdez Casás, AIMEN Research Center, Spain

MXenes are layered transition metal carbides, carbonitrides, and nitrides produced from layered ternary materials known as MAX phases by selective etching their A-layers. SAFARI project main objective is the SSbD production and utilization of MXenes, covering the whole supply chain, starting from the precursors preparation, MXenes production and functionalization and going to end applications. MAX phases production route based on Spark Plasma Sintering (SPS) and High Energy Ball Milling (HEBM) combination has been adopted to produce 2 MAX phases: Ti₃AlC₂ and Cr₂AlC. Next, Ti₃C₂ and Cr₂C MXenes can be obtained from their corresponding MAX phases via High Frequency Acoustic Emission (HFAE). Chemical digestion (CD) and microwave (MW) as sustainable source of heat were used for the MAX phases etching.

The objective of this study is to determine the environmental impacts of MAX phases and MXenes production via LCA assessment in order to identify hotspots and improvement opportunities, thereby supporting the innovation process. An LCA was carried out following ILCD handbook and ISO14040 methodology. The software SimaPro v9.6 with the database Ecoinvent 3.10 and the ILCD 2011 Midpoint+ method were used. The functional unit was 1 kg of manufactured material (MAX phase or MXene), and the scope was Cradle-to-Gate. Primary data obtained from the production process data (inputs, outputs, wastes and energy requirements) was used. Regarding MAX phases, Cr₂AlC has a greater environmental impact than Ti₃AlC₂ (1.7 times higher). In both MAX Phases, most of the environmental impact is located in the category Human toxicity, cancer effects (1.8 times higher in Cr₂AlC), followed by Freshwater ecotoxicity (1.3 times higher in Cr₂AlC), Human toxicity, non-cancer effects (1.2 times higher in Ti₃AlC₂) and Ionizing radiation HH (same for Cr₂AlC and Ti₃AlC₂). Regarding MXenes, the environmental impact of Ti₃C₂ CD and Ti₃C₂ MW is very similar. In both cases, the order of the categories with the highest environmental impact remains practically the same as in the case of the MAX Phase, except that Ionizing radiation HH is slightly higher (1 point) than Human toxicity, non-cancer effects. The study highlights the great importance that the choice of the metal to be used in the MAX Phase has in relation to the environmental impacts that the production process of these new materials can cause and also the big impacts that MAX Phases and MXenes production processes have on human health.

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5.02.P-Mo430 The Role of SSbD for Pesticides Substitution

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Safe and sustainable-by-design (SSbD) is an emerging concept that can be applied for evaluating different pest control options. However, defining functionally equivalent scenarios for applying SSbD to substitute hazardous pesticides is challenging, especially for contrasting field operations with supply chain impacts in SSbD step 4 on environmental performance. To address this question, we propose a framework to generate functionally equivalent substitution scenarios and test it in a real-world case study. We define conditions for substituting a set of authorized pesticides targeting the same pests. A set of matching tables is integrated into a substitution matrix to compare scenarios. Using this matrix, we compared four pesticide scenarios (Oxathiapiprolin, Laminarin, Folpet + Mandipropamid, and Copper (II) hydroxide) applied in grapes/vine in Europe to prevent Downy mildew, representing chemical, natural, and copper-based fungicides. Life cycle assessment is applied to quantify environmental impacts (SSbD step 4) of all scenarios, and normalization is applied to rank scenarios. Our results show that emissions from field operations of Folpet and Mandipropamid dominate human health impacts. They cause total human toxicity impacts of 0.0014 DALY or 12 hours human life-time lost per 1 hectare application. Pesticide supply chain emissions dominate marine ecotoxicity impacts due to the emissions of 55-62% Aluminium (III), 15-36% Barium (II), and 7-11% Strontium (II). Oxathiapiprolin is identified as overall best-in-class fungicide alternative, followed by Laminarin. Oxathiapiprolin causes 0.2% of overall single-score impact compared with Copper (II) hydroxide. Copper (II) hydroxide shows the highest impacts across alternatives for some supply-chain related impact categories (e.g. human toxicity), while Folpet + Mandipropamid dominate human toxicity and ecotoxicity from field operations. The two latter pesticide scenarios should be considered as candidates for substitution, where environmental conditions, pest resistance and other relevant aspects allow application of proposed low-impact alternatives. The results show that environmental performance at the level of scenario can be improved up to seven orders of magnitude in

our case study. Our substitution scenarios will have to consider additional performance criterias (e.g., hazard, worker/bystander health, equivalent efficacy, antagonistic effect, pesticide resistance) to become a viable element in SSbD.

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5.02.P-Mo431 Comparative Assessment of Early-Stage Social and Critical Raw Material Assessment in the SSbD Framework: Addressing Current Generic Databases

Ashrakat Hamed, Jonas Hoffmann and Andreas Ciroth, GreenDelta GmbH, Germany

As part of the Safe and Sustainable by Design (SSbD) framework, socio-economic assessments provide a holistic approach by ensuring socio-economic and environmental impacts are considered from the early development stages. However, such assessments, particularly for low Technology Readiness Level (TRL) materials and technologies, remain uncommon and challenging due to limited or unreliable data. Existing social databases, such as PSILCA, are based on current knowledge and can serve as a preliminary tool to identify potential social hotspots. Similarly, tracking data from background databases likeecoinvent, which contain material usage information, offers a useful starting point for assessing critical raw material use in supply chains. In this study, a preliminary comparative assessment was conducted between petroleum-based lubricant and bio-based lubricant (soybean) in terms of social performance and critical raw material consumption. Results reveal that petroleum-based lubricants exhibit higher opportunities for economic development (10% High Opportunity) compared to bio-based lubricants (8% High Opportunity). However, petroleum-based lubricants also present significantly higher social risks, particularly in themes such as "Fair Salary," where up to 52% of the impacts was classified as Very High Risk. Additionally, a comparison of critical raw material consumption shows that soy-based lubricants have a higher environmental impact, with soy-based materials exhibiting substantially higher values in the SH2E Criticality Indicator compared to their petroleum-based counterparts. These findings underscore a key limitation of relying on generic databases for low TRL materials, as the bio-based lubricant was linked to inaccurate supply chain data, which contributed to unreliable impact calculations. This highlights the challenges of using such databases to perform accurate assessments at early stages of technology development, emphasizing the need for more precise and up-to-date data to guide decision-making.

5.02.P-Mo432 Visualization of Nitrogen Flow in Japan Using IDEA Database

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In the concept of Planetary Boundaries, excessive atmospheric nitrogen consumption caused by population increase and economic growth has received widespread attention during recent years. This is due to the use of atmospheric nitrogen, which is released back into the atmosphere and water as reactive nitrogen (ex, NO_x, total nitrogen) after it has been used in various processes. Therefore, it is important to analyze the nitrogen flow to reduce the burden on the environment. In previous LCA research, reactive nitrogen has been targeted as elementary flow regarding as environmental burden substance. On the other hand, input data including atmospheric nitrogen, **NH₃** and so on have not been targeted as elementary flow regarding as resource or environmental burden substance.

Additionally, technologies which aim to recycle reactive nitrogen into atmospheric nitrogen for reducing environmental burden have been developed in recent years. In this case, we need to analyze environmental burden in total. Hence, we need not only amount of reactive nitrogen as output data, but also amount of nitrogen as input data. At the same time, we need to confirm the nitrogen balance between input and output to verify the validity of the entire mass balance process because it is significant to reduce the environmental burden in entirety.

In this presentation, we will show the results of nitrogen flow in Japan using IDEA database. We developed the nitrogen input and output amount for each process data in IDEA and analyzed total nitrogen amount of target year, 2015.

5.02.P-Mo433 Integrated Freshwater Risks of Nano-Encapsulated Imidacloprid Versus Its Conventional Counterpart: A Life Cycle Perspective

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The use of nanopesticides is becoming increasingly widespread; however, current knowledge does not thoroughly address their environmental impacts throughout the life cycle while considering nano-specific properties and environmentally relevant scenarios. To bridge this knowledge gap, we first assessed the cradle-to-gate environmental impacts of imidacloprid (IMI) and nano-encapsulated imidacloprid (nano-

IMI) manufacturing via a life cycle assessment. By coupling the USEtox and SimpleBox/SimpleBox4Nano models, we derived ecotoxicity-characterization factors and impact scores for both IMI and nano-IMI, enabling the quantification of ecological risks associated with their end-of-life emissions. The relative ecological risks of nano-IMI during production (4,631 CTUe) were approximately four times higher than those of IMI (1,177 CTUe). Conversely, the freshwater risks associated with end-of-life nano-IMI release ($0.012 \times 6.93 \times 10^4$ CTUe) were at least one order of magnitude lower than those of IMI ($1.59 \times 10^3 \times 6.13 \times 10^6$ CTUe), considering the impacts of rainfall, toxicity data selection, fate, and transport. Under identical rainfall conditions, nano-IMI exhibited substantially reduced integrated freshwater risk compared with IMI, showing potential as an alternative to conventional IMI. This study provides a novel approach for assessing engineered nanomaterial alternatives considering various possible scenarios and offers valuable insights into the risk assessment of nanopesticides throughout their lifecycles for the first time.

5.02.P-Mo434 Life Cycle Assessment of Electronic, Electric and Nonelectric Detonators; A Site-Specific Case for Czech Republic

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The widespread use of detonators across industries such as construction and mining introduces significant environmental risks throughout all of their life cycle stages, creating a need to understand and mitigate their generated environmental impacts. Although there have been studies addressing Life Cycle Assessment (LCA) of specific explosives within detonators (e.g. trinitrotoluene, hexogen or octogen), a study about comprehensive environmental evaluation of detonators as whole product is missing. Our study addresses this issue by evaluating the environmental footprints of three most commercially used types of detonators electronic, electric, and non-electric. The electronic detonator is distinguished by e.g. high variability detonation timing and is mostly used for detonations in quarries, metal and nonmetal mines or construction sites. Thanks to its high initiation strength, the electric detonator is suitable for blasting of trenches, shafts or raises. The nonelectric detonator provides excellent water resistance making it suitable for explosions of cap sensitive emulsions. The primary objectives are: 1. to evaluate the environmental impacts of the detonators life cycle phases, 2. to identify which detonator type poses the greatest environmental burden and 3. to propose strategies leading to potential environmental burden mitigation. The LCA includes traditional stages such as comprehensive Life Cycle Inventory (LCI), followed by Life Cycle Impact Assessment (LCIA) according to EN 15804+A2 Environmental Footprint (EF 3.1) with a final interpretation. As functional unit is chosen one piece of detonator with system boundaries set to cradle to grave. Results indicate that the detonators exhibit the highest environmental impacts for categories Freshwater Ecotoxicity, Global Warming Potential and Abiotic Fossil Depletion, with the electronic detonator having the most considerable impact. Based on these findings, we recommend specific measures, such as adopting metals with recycled content, bio-based and -degradable plastics or alternative fuels, to support more sustainable detonator production and usage. However, the measures need to be adopted after individual proper fit-to-purpose analysis (analysing economic feasibility, technological properties or availability).

5.02.P-Mo435 A Comprehensive Life Cycle Assessment of Carbon Capture Enabled by Metal-Organic-Framework CALF-20

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Metal-Organic Frameworks (MOFs) are crystalline materials made up of metal ions or clusters bonded to rigid organic molecules, forming structures that can be one-, two-, or three-dimensional and often porous. These frameworks are built using coordination bonds between metal ions or clusters and multidentate organic linkers. MOFs are well-known for their large surface areas, adjustable porosity, versatile shapes, high stability, and multifunctional properties, making them suitable for applications such as gas storage and carbon capture. However, there are notable drawbacks, such as concerns about their stability during use and the risk of releasing toxic metal ions or organic components into the environment as they degrade. The environmental impact of MOFs is an important issue, as their long-term behavior and how their components interact with ecosystems are not fully understood. One significant weakness of many MOFs is their stability in water; even small amounts of moisture can affect their structural integrity and porosity. Consequently, the potential for bioaccumulation and toxicity raises serious questions about the safety of using MOFs in practical applications, highlighting the need for further research into their ecological effects.

In this work, by following the Safe and Sustainable by Design principles as implemented in the Triple-S methodology, we employed Life Cycle Assessment (LCA) to focus our comparisons specifically on a Zn-based MOF named Calgary Framework 20 (CALF-20). The objective of this LCA is to evaluate the environmental impact associated with the synthesis of Zinc MOFs, identify hotspots within the life cycle, and optimize the production process while considering both performance and yield. The scope of the LCA encompasses two distinct boundaries: a cradle-to-gate assessment with a functional unit of 1 kg of Zn MOF, and a cradle-to-grave analysis focused on the goal of removing 1 ton of CO₂. For comparison within the same design space, we examined five different MOFs such as aluminum fumarate and other CALFs utilized in pulp and paper and cement industry. Additionally, the comparison of different synthesis routes involving solvents is excluded from scope, as the solvents do not align with sustainable practices. By employing LCA methodology, this study aims to provide comprehensive insights into the sustainability and efficiency of Zn MOF production, ultimately contributing to the advancement of environmentally friendly materials.

5.02.P-Mo436 Dual Importance of Method and Data for Robust Sustainability Assessment: Integrating Life Cycle Assessment and Principles of Green Chemistry to Identify More Sustainable Cosmetic Product Ingredients

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Frameworks for guiding sustainable product design draw from various fields, and as a result, use varied data, models and metrics. The Sustainable Innovation Profiler (SIP) is a new software tool that leverages life cycle assessment using the product environmental footprint (PEF) framework and the principles of green chemistry as two alternative lenses through which to evaluate sustainability of chemical substances used as ingredients in cosmetics and personal care product formulas. This tool, described in a separate poster, was created to enable product developers to compare alternative product designs using multiple metrics to avoid blind spots and improve decision making for sustainable innovation.

Robust sustainability assessment relies equally on using scientifically sound methodology and the best available data. To avoid preventable regrettable substitution errors, where more sustainable ingredients are erroneously replaced by less sustainable ones due to outdated or missing data, it is crucial to carefully curate the databases underlying decision tools like the SIP, used to guide product development.

Sustainability assessments generated by the SIP for the formula portion of a cosmetic product require accurate data on the ingredient composition in the formula, environmental sustainability characteristics of ingredients (e.g., environmental degradation rates, carbon emissions, results of aquatic toxicity studies), and the manner of use of the product (i.e., rinsed off after use or not).

This poster shows results of the tool's parallel metrics used to evaluate sustainability for several sets of alternative ingredients (e.g., ultraviolet filter ingredients used in sun protection products). Examples include a demonstration of the higher risk of regrettable substitution errors for ingredients that are under active study by academic researchers, and for which new sustainability data (e.g., from ecotoxicological or environmental fate studies) are being generated and published at a rapid rate. Results also show where trade-offs among the different sustainability metrics for alternative ingredients occur, and for some alternatives, where such trade-offs can be avoided. This work demonstrates the value of using carefully curated data, models, and metrics to evaluate and select among potential alternative ingredients early in the product design process to create cosmetic products with a superior environmental sustainability profile.

5.03 Prospective Life Cycle Assessment for Safe and Sustainable by Design (SSbD) Innovations

5.03.T-01 Prospective Life Cycle Assessment Framework for Continuous Pharmaceutical Production: Upscaling from Lab Scale

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Continuous Flow Production (CFP) has emerged as a sustainable alternative to batch production in the pharmaceutical industry, offering reduced waste, improved heat and mass transfer, and higher yields. However, scaling up lab-scale CFP processes for industrial-level sustainability assessments remains challenging due to data uncertainties and the lack of standardized procedures. This study addresses these challenges by developing a structured framework for scaling up continuous pharmaceutical manufacturing processes for prospective Life Cycle Assessment (LCA). The proposed framework employs a stepwise

approach, beginning with a comprehensive literature review to extract relevant lab-scale data, such as synthesis steps, material inputs, and yields. These steps are upscaled using geometric and kinematic similarity, empirical scaling laws, and thermal modelling, supported by collaboration with industrial partners and insights from recent commercial production practices. Logical scenarios were defined to address uncertainties, ensuring flexibility in modelling performance variations in energy use, resource consumption, and emissions. The framework, encompassing nine unit operations and 13 equipment types, enables the estimation of Life Cycle Inventory (LCI) data with minimal resources. This study is the first to develop an estimation method for industrial-scale pharmaceutical production, including product formulation, using optimized commercial equipment designs and performance data. It offers LCA practitioners a practical and efficient tool to evaluate the sustainability of pharmaceutical production processes.

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5.03.T-02 Prospective Life Cycle Assessment of Itaconic Acid Production: Scaling-up and Future-Oriented Scenarios

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Carbon capture and utilisation (CCU) technologies can be essential to decarbonise productive sectors and achieve European and global climate goals. Carbon dioxide (CO₂) valorisation into chemicals poses several challenges, including the development of innovative, competitive and resource-efficient processes within environmental constraints. Genetically engineered microorganisms have shown the potential for alternative feedstock bioconversion into platform chemicals, including itaconic acid (IA) production from methanol using *Komagataella phaffii*. However, technological maturity and scaling-up uncertainties are a few of the barriers encountered when assessing the sustainability of such emergent technologies at early development stages. The complexity is further increased when attempting to include future scenarios that depict decarbonised supply chains. To this end, Prospective Life Cycle Assessment (p-LCA) is a tool that addresses scaling-up challenges of low technology readiness level (TRL) technologies whilst considering future scenarios for energy and electricity markets.

This study presents the cradle-to-grave p-LCA for IA production using an engineered *K. phaffii* strain and two sources of methanol as carbon feedstock: fossil and CO₂-based. The latter was modelled from captured CO₂ and electrochemically produced hydrogen. The approach for the Life Cycle Inventory followed foreground process modelling from laboratory-scale data using SuperPro designer and an engineering scale-up framework. Prospective modelling followed the SSP2-RCPI.9 pathway for 2020, 2030 and 2050 according to the IMAGE Integrated Assessment Model. The premise tool was used to transform the background database (ecoinvent 3.10 cut-off) within Brightway 2.5. The assessment was conducted according to the EF v3.1 impact method. This assessment included the comparison against a bio-based IA benchmark.

Preliminary p-LCA results showed an improved environmental performance for the CO₂-MeOH pathway, underlining the relevance of electricity decarbonisation for CO₂ valorisation processes. The comparative against bio-based IA reinforced research efforts to develop strains with improved productivity metrics and low-pH resistance for a competitive CCU technology. Altogether, the study remarked the role of future-oriented scenarios combined with benchmark analysis to support decision making towards the fulfillment of decarbonisation goals within a Safe and Sustainable by Design framework.

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5.03.T-03 Designing a Future Plastic Industry Under the Triple Planetary Crisis

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The global plastic industry, currently fossil-based and linear, contributes to the triple planetary crisis of climate change, biodiversity loss, and pollution. This study presents an integrated optimization model

(PolyLOP) to design and assess future scenarios for the global plastic industry, uniquely addressing all aspects of the triple planetary crisis.

Our findings demonstrate that achieving net-zero greenhouse gas emissions in the plastic industry by 2050 is feasible but requires implementing multiple strategies simultaneously: utilizing alternative feedstocks (lignocellulose residues and captured CO₂), maximizing mechanical and chemical recycling, and deploying carbon capture and storage (CCS) for biogenic CO₂ emissions. However, this transition creates significant trade-offs. The net-zero scenario results in 20% higher particulate matter-related health impacts compared to a fossil-linear scenario, primarily due to ammonia emissions from biomass cultivation. Additionally, it puts 0.42% of global species at risk of extinction due to increased biomass utilization. The transition faces several critical challenges. Net-zero becomes unfeasible if biomass availability falls below 90% of the projected potential in 2050. The preferred production pathway at low electricity carbon footprint using CO₂ feedstock requires substantial hydrogen production, pushing industry electricity consumption to nearly 10 PWh over one-third of current global production. Furthermore, the industry faces a lock-in situation due to existing fossil-based infrastructure, potentially emitting an extra 0.35-0.55 Gt CO₂-eq by 2050.

Our analysis reveals that reducing plastic demand is crucial; net-zero emissions become unattainable when production exceeds 1.2 Gt. Regional variations in resource availability and environmental impacts necessitate tailored approaches, with some regions bearing larger biodiversity losses and health burdens while contributing to reduced global emissions. International collaboration through cross-border resource exchange and technology transfer is essential to bridge the gap between regional capacities and global sustainability strategies.

This study illuminates the complex trade-offs between local and global environmental benefits and impacts, enabling informed decision-making and targeted mitigation strategies for the plastic industry's sustainable transition.

5.03.T-04 Material Shortage: A Barrier to Clean Energy and Climate Goals

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The importance of clean energy technologies (CETs) in mitigating greenhouse gas (GHG) emissions is well-documented. However, the feasibility of developing CETs timely at the required scales and the consequences of potential shortfalls in their deployment remains underexplored.

This study assesses the achievable capacities of some CETs considering the availability of 36 materials required for their construction. CETs assessed include stationary and electric vehicle batteries, photovoltaic panels, wind turbines, and concentrated solar power (CSP) systems. We depart from 25 CET capacity projections sourced from eight different Integrated Assessment Models (IAMs). These projections, aligned with the Paris Agreement, are translated into material requirements using material intensities for different CETs, and compared with material availabilities obtained from our optimization model. The optimization model aims to minimize the gap between IAM projections and the capacity of CETs that can be realistically built according to estimates on material supply rates.

Shortfalls in CETs will result in additional GHG emissions if unmet capacities are replaced by equivalent business-as-usual services, such as grid electricity or diesel vehicles. Hence, we resort to prospective life cycle assessment (LCA) to calculate GHG emissions that would result from gaps in CET capacities between 2020 and 2050, and ultimately translate these into the equivalent of global warming.

Acknowledging that the impact of these shortfalls in CETs will be dictated by the evolution of the markets, we consider two extreme scenarios. The Current Policy (CP) scenario, reflecting the second Shared Socioeconomic Pathway (SSP2) and Representative Concentration Pathway 3.4 (RCP3.4), projects warming beyond 2 °C, providing an upper bound on the additional GHG emissions. The Net Zero (NZ) scenario, aligned with SSP1 and RCP1.9, limits warming to 1.5 °C and represents a lower bound on the additional GHGs.

We identified significant shortages, with developed capacities falling short by as much as 94% for batteries and PV panels. Depending on the scenario, these shortfalls could contribute to additional warming of 0.06 to 0.95 °C. This underscores the need to incorporate material availability constraints into IAMs to obtain more realistic estimates of CETs and, ultimately, support evidence-based policymaking for a seamless transition to sustainable energy systems in line with the Paris Agreement.

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5.03.T-05 How to Decarbonise the Steel Industry? A Prospective Life Cycle Assessment to Compare Different Technological Solutions

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The iron and steel sector ranks first in CO₂ emissions and second in energy consumption amongst heavy industries. This study focusses on three decarbonisation technologies for the steel industry, all under development at technological readiness level (TRL) 7: INITIATE, DISPLACE and CASOH. The INITIATE technology uses sorption-enhanced water gas shift (SEWGS) technology to transform blast furnace gas (BFG) and basic oxygen furnace (BOFG) into a suitable feedstock for ammonia and urea production. All CO₂ that is left after producing urea can be geologically stored². DISPLACE, a carbon capture technology using similar hydrotalcite-based adsorbents, uses displacement adsorption to capture CO₂ from various flue gasses from the steel production (i.e., sinter plant, hot stoves and reheating ovens)³. Lastly, CASOH, uses calcium-enhanced looping to capture CO₂ from BFG, while simultaneously creating an energy rich hydrogen fuel stream³. The captured CO₂ in DISPLACE and CASOH is geologically stored.

The goal of the study is to assess the decarbonisation potential of implementing a single or a combination of these technologies in steelmaking on industrial scale. Further, environmental hotspots on human health damage, ecosystem damage, resource scarcity and climate change specifically are identified to provide recommendations towards industrial scale application while minimizing the environmental impact.

We looked at conventional steel production (i.e., base case) and steel production with amine carbon capture technology and storage (i.e., reference case). Life cycle inventory foreground data was modelled in Aspen plus, with background data obtained from a prospective database based on ecoinvent 3.9.1 and IMAGE (SSP2) future scenarios made using premise.

It was found that CASOH in combination with DISPLACE and the INITIATE technology can achieve large reductions in climate change impacts compared to conventional steel production and steel production with amine carbon capture. Specifically, reductions of 1116 kg CO₂-eq/ton HRC steel to 1283 kg CO₂-eq/ton HRC steel compared to the base case are found, respectively. These technologies can aid in the decarbonisation of the steel industry and fertilizer industry.

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5.03.P Prospective Life Cycle Assessment for Safe and Sustainable by Design (SSbD) Innovations

5.03.P-Mo437 SSbD Approach to Enhance the Environmental Sustainability of Industrial Washing Processes through Usage Biodegradable Detergent Based on Life Cycle Assessment

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Industrial washing processes in automated production face a number of environmental challenges including significant wastewater pollution and consumption of non-renewable resources. There is a need for safe and sustainable alternatives to conventional detergents that maintain cleaning efficiency but minimise negative environmental impacts. The study focused on applying the Safe and Sustainable-by-Design (SSbD) approach to optimise washing processes traditionally characterised by high energy, water consumption and the use of chemically aggressive detergents. The main objective of the study is to reduce the environmental footprint of washing processes in automated manufacturing by introducing a biodegradable detergent and assessing its impact through Life Cycle Assessment (LCA) methodology in Step 4 of SSbD approach.

In this study, the washing process using a new generation biodegradable detergent was modelled and compared with conventional detergents using the LCA method (ISO 14040/44). Step 4 of the SSbD approach was applied to assess the environmental sustainability and safety of the selected solution. The analysis included an examination of the environmental impact of washing processes with different

detergent use, mainly during the use phase of the compared products. The study assessed key indicators such as carbon footprint, water footprint, ecotoxicity and energy consumption.

Preliminary data showed that transition to a biodegradable detergent resulted in a reduced carbon footprint compared to conventional counterparts. Ecotoxicity was reduced due to the improved formulation of the detergent and its biodegradability reduced the load on wastewater treatment systems. Additionally, there was a reduction in energy consumption while maintaining equipment cleaning standards.

The study demonstrates that the use of biodegradable detergents based on the SSbD framework significantly reduces the environmental footprint of the washing processes while maintaining high cleaning efficiency. The results highlight the potential of integrating SSbD into different operational processes as a key strategy to achieve environmental sustainability. These findings are applicable for scaling to other industries with high environmental burdens, promoting the principles of circular economy and sustainable development.

5.03.P-Mo438 Prospective Life Cycle Assessment of Advanced Materials – The Case Study of Graphene Oxide in Drinking Water Filters

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The European Commission, in its Recommendation for the implementation of a framework for safe and sustainable by design (European Commission 2022), advises to use Life Cycle Assessment (LCA) to evaluate the environmental sustainability of chemicals and materials. However, its applicability to Advanced Materials (AdMa) can be challenging due to lack of data, pertaining to the novelty of these materials and their production processes. In this practical case study, we are exploring how prospective LCA can be implemented to assess the environmental impacts of producing a polysulfone graphene oxide (PSU-GO) membrane used in drinking water filters.

The lifecycle inventory was built on exchanges of information with the filters producer, enabling the use of first-hand foreground data for a contribution. Using Environmental Footprint v3.1 and Impact World+ Midpoint v2.0.1, and as expected for inventories created for early stages of product development, results showed that equipment contributed to more than 95% of the process impacts for all impact categories. When excluding the contribution of equipment to the impact assessment, electricity and the raw material N-methyl-2-pyrrolidone (NMP) were the main contributors (e.g. 45% each of impacts on freshwater ecotoxicity using EF v3.1).

In a next step, to better approximate material and energy uses and evaluate the contribution of equipment to the impacts, a methodology is being developed to upscale this pilot process to an industrial scale. Based on manual calculations and use of proxies, the impacts of the foreseen industrial production process are compared to those of the same filtration membrane without GO. To ensure the functionality is consistent in both systems compared, activated carbon will be fictively added to the filtration membrane not containing GO.

The potential implications of these results on the design and development of the industrial process are discussed, as well as learnings on the applicability of prospective LCA for SSbD.

5.03.P-Mo439 Prospective Life Cycle Assessment of a Modular Shared Autonomous Vehicle Production Phase

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The transportation sector's greenhouse gas emissions have driven research into sustainable vehicle production. This study presents a Life Cycle Assessment (LCA) of a prototype Modular Shared Autonomous Vehicle (MSAV), focusing specifically on its production phase to explore environmental impacts relative to conventional electric vehicles (EVs). By concentrating on production impacts in the early stages of MSAV development, we aim to inform strategies incorporating Safe and Sustainable by Design principles into emerging vehicle technologies.

This case study utilizes primary data on materials and manufacturing methods unique to the MSAV, which is rarely available in vehicle LCA studies. We apply prospective LCA methods to develop future production scenarios that include renewable energy adoption, advances in material efficiency, and emerging manufacturing techniques like additive manufacturing. In particular, we model improvements in high-impact materials such as carbon fiber and additional electronics used in the autonomous driving system, both of which significantly influence the environmental footprint of the MSAV's production phase. As recycling options, manufacturing efficiencies, and production technologies evolve to higher Technology Readiness Levels (TRL), these materials and components are expected to become more

sustainable.

Preliminary results indicate that, due to added electronics and specialized materials, the MSAV's production phase currently has a higher environmental impact than conventional EVs. However, scenario modeling suggests that future advancements in manufacturing methods, including additive manufacturing, as well as improved energy sourcing and material innovation, could significantly reduce the environmental impacts associated with MSAV production.

Our findings highlight the potential of forward-looking production strategies to align MSAV design with Safe and Sustainable by Design principles. By providing insights into future production scenarios and design improvements to reduce environmental impacts, this study acts as a foundation for policymakers, manufacturers, and researchers dedicated to advancing sustainable urban mobility. This work emphasizes the critical role of early design and production choices in shaping a cleaner, more resilient future in transportation.

5.03.P-Mo440 Ex-ante Life Cycle Assessment of Lipids Extraction from Insect Biomass

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Research on insects as an alternative food source has been developing in the last decade. They offer advantages such as a high nutritional value and protein content, with a lower environmental impact compared to traditional livestock.

The use of insect biomass, however, is not relegated to food applications as it can be a viable substitute as an oil source for cosmetic applications, having a similar chemical composition to currently used vegetable oils. In this context, the PINSO project is a research endeavor from Thomas More and KU Leuven that aims to demonstrate the feasibility of insect lipid production on a pilot scale and the technological and economic viability of further scaling.

Environmental sustainability assessment is an integral part of the process, conducted through the Life Cycle Assessment methodology according to ISO 14040 standards.

The research project comprises two phases: the LCA of the pilot plant in Geel (BE), with lab-scale oil extraction from *Tenebrio molitor* larvae, and a conceptual scale-up of the process and estimate of the associated energy and mass balances, to then perform an ex-ante sustainability assessment, using a hybrid scale-up framework from existing literature.

Results may be relevant in offering insight on environmental hotspots of the process, to suggest further research on the optimization and industrial applicability of the process.

Furthermore, uncertainty of results is discussed and suggestions for further research are presented.

5.03.P-Mo441 Revealing Biodiversity Impacts of Land Use Intensification: A Spatio-temporally resolved Global Assessment (2005–2019)

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Land use is the key driver of biodiversity loss, yet its full impacts remain underestimated due to the lack of consideration for land use intensity and the absence of spatio-temporally resolved inventory data [1]. This study assesses spatially-resolved biodiversity loss from land use intensification from 2005 to 2019. We identify critical biodiversity hotspots and quantify the effect of land use intensity on global extinction rate. A global dataset of land use was compiled from satellite-based sources, classifying land use into three levels of intensification (minimal, light, and intense) across various land types, including crops, pasture, plantations, managed forests, and urban areas. Using characterization factors (CFs) accounting for land-use intensities and five species groups, we estimated extinction rates on ecoregion-level and compared our results with UNEP-SETAC methods. Preliminary results show that land-related biodiversity loss has accelerated over the 2005-2019 period, with a global extinction rate reaching 17% in 2015. This is almost two times higher compared to previous studies that did not account for spatially-resolved land use intensification. Hotspots of biodiversity loss include Brazil, Mexico, Southeast Asia, and East Africa, with significant increased extinction rates due to intensified use of pastures, plantations, and managed forests. These findings underscore the importance of incorporating land use intensities into biodiversity impact assessments, revealing the urgency of adopting more sustainable land management practices. By integrating spatio-temporally resolved remote sensing data and applying refined characterization factors,

this study offers valuable insights for improving conservation strategies and informing policies to mitigate biodiversity loss in the face of global food demand and environmental pressures.

5.03.P-Mo442 Influence of Different Uncertainties Types in Prospective LCA: A Case Study on Organic Active Materials for Flow Batteries

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The sustainability and safety of stationary energy storage systems (ESS) such as redox flow batteries (RFB) have recently become an issue, particularly since ESS are considered to play an essential role in renewable energy transitions. Organic active materials (for redox-flow batteries, ORFB) produced from industrial by-products, e.g. lignin, may be advantageous regarding sustainability considerations. However, this cannot be taken for granted and given the currently low technology readiness level (TRL) and, consequently, high uncertainties prevailing, continuous evaluation of ESS options seems highly relevant. Since the provision of point estimates for environmental impact (EI) comparisons of battery alternatives may lead to poor decision-making towards Safe and Sustainability-by-Design (SSbD) innovations, different types of uncertainties need to be identified, systematically addressed, and communicated. Therefore, the aim of this work is to identify and characterize uncertainties present in ESS (and particularly ORFB) development and to take these into account with a prospective analysis approach. In setting up the production-phase inventories, related uncertainties are considered in several ways which are addressed and discussed in this work. In addition, since the operation and EoL of batteries are the phases associated with the most uncertainties, an equation-based model is proposed for the calculation of EIs under consideration of specific use-phase-related technical performance parameter settings for respective ESS systems and application scenarios. Preliminary cradle-to-use Monte-Carlo results depicting potential use-phase-induced ranges of EIs (Global Warming Potential, GWP) indicated that (uncertainties regarding) battery cycle-life and (future) energy sources used for charging/discharging showed to be substantial for the potential extent (ranges) of use-phase induced GWP impacts. This way forward should enable more substantiated comparisons between ESS options and support decision-making on related SSbD innovations. A better and more systemic understanding of potential EIs of ESS technologies that are currently (partly) at lower TRLs, identification of hotspots, key levers, and trade-offs, as well as grasping how prevailing uncertainties change in the course of the technology development process are further aims followed. As a starting point, this study provides suggestions on how these issues could be tackled and presents first results.

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5.03.P-Mo443 Implementing Prospective Life Cycle Assessment in Process Systems Engineering: A Case Study of Bio-Based Acrylic Acid Production from Wheat Straw Through Thermo-Mechanical Pulp Mill Side Stream

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Acrylic acid is a valuable platform chemical with significant potential for conversion into commercially essential products, including acrylates, sodium polyacrylate, polyacrylic acid, acrylamide, and polyacrylamide. Currently, the primary method for industrial acrylic acid production involves catalytic oxidation of fossil-based propylene, a process associated with stringent safety requirements and substantial environmental impact. Alternatively, bio-based acrylic acid production from wheat straw through thermo-mechanical pulp mill side streams offers a more sustainable pathway. A prospective life cycle assessment (pLCA) is an essential approach for early-stage evaluation of this innovative biochemical pathway, allowing for a comparative analysis between bio-based and fossil-based acrylic acid production. This assessment provides valuable insights to guide and support stakeholders and decision-makers in advancing sustainable production methods. The study aims to identify environmental hotspots during the initial development phase, using a cradle-to-gate system boundary and a functional unit of 1 kg of acrylic acid. Two-time horizons are considered: the present year, 2024, and future years up to 2050, when industrial-scale implementation of this biochemical pathway is anticipated.

The assessment incorporates three prospective scenarios, aligned with the Paris Agreement's climate

targets for energy system development, based on scenarios of an integrated assessment model (IAM), the REMIND model, which are the i.e., REMIND-SSP2-Base (projecting a 3.5°C temperature rise by century s end), PKBudg1150 (targeting below 2°C by 2100), and PKBudg500 (aiming for a cap below 1.5°C by 2100), corresponding to milestones in 2030, 2040, and 2050. Using the ReCiPe 2016 v.1.03 life cycle impact assessment (LCIA) method, this study performs a hierarchical midpoint assessment to quantify environmental impacts, focusing on global warming potential (GWP) measured in CO₂ equivalents. Results reveal that the primary contributor to GWP is the energy-intensive evaporation process required to concentrate the diluted side stream, marking it as an environmental hotspot. Findings also indicate that as future energy production becomes more sustainable, the viability of bio-based acrylic acid production improves. Overall, bio-based acrylic acid demonstrates a lower GWP than fossil-based acrylic acid for both current and future scenarios.

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5.03.P-Mo444 Generative Multi-Agent Framework for Prospective Life Cycle Assessment Aligned with Safe and Sustainable by Design Principles

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This research presents the "CarbonQi" framework, a generative AI-driven approach for prospective life cycle assessment (LCA) of emerging technologies, with a focus on battery technologies. By leveraging generative AI's high-level reasoning and dynamic modeling capabilities, the framework addresses the challenges of incomplete data and rapid technological changes in early-stage development. The modular design enables flexible updates, providing real-time carbon footprint predictions, and enhancing decision-making for low-carbon strategies. This innovative method supports Safe and Sustainable by Design (SSbD) principles by enabling early environmental impact assessments, ultimately guiding the design of more sustainable technologies.

5.03.P-Mo445 ProScale-E: Ecotoxicity Potential Assessment, from Method Development to Application

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This abstract presents the application of the recently developed ProScale-E method for ecotoxicity potential assessment within a case study. Previously, the ProScale method covering direct exposure related human toxicity potential, here referred to as ProScale-H, was established as a method based on a straightforward mathematical formula, aligned with REACH as much as possible, and requiring little to moderate substance data while still providing meaningful results. From collaborative work among participating consortium members, the ProScale-E method was recently developed according to similar principles and presented to the whole ProScale-E consortium in May 2024. ProScale-E is proposed as a promising method for safe and sustainable by design (SSbD) chemicals assessment in early innovation stages. In the current work, the ProScale-E method was applied within a case study simulating impact assessments at early innovation stages, i.e. with limited information available, for instance on hazard data and chemical emissions. Here, the authors focused on assessing the ecotoxicity potential of bisphenol A (BPA) based polycarbonate within a cradle-to-gate system (which may be extended to cradle-to-grave).

Within this case study work, the product system of BPA-based polycarbonate was modelled with ProScale-E. The results of a ProScale-E assessment correspond to a hazard- and degradation-based score giving quantitative information on direct chemical risks for the environment. According to default worst case release factors based on Environmental Release Categories (ERCs) and Specific ERCs (SpERCs) (as proposed by ECHA for environmental exposure assessment within REACH), the authors modelled emissions to air, water and soil. The preliminary results allow to identify the hotspots: chemical emissions with highest ProScale-E score, resulting in a large contribution to the overall impacts.

The ProScale-E method for ecotoxicity potential assessment was applied to BPA-based polycarbonate. The model could successfully translate REACH data into ecotoxicity potential of modelled emissions throughout the life cycle of the studied product. Worst case release factors derived from ERCs and SpERCs allowed for a rough modelling of substances release to air, water and soil. It is here suggested that by applying this method the ProScaleE practitioner can easily identify ecotoxicity hotspots.

5.03.P-Mo446 Advancing Maritime and Aviation Fuels Through Prospective LCA: A Safe and Sustainable by Design Approach

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The maritime and aviation sectors significantly contribute to global greenhouse gas (GHG) emissions, accounting for approximately 3% and 2%, respectively. In response, both industries are pursuing ambitious decarbonization strategies. The International Maritime Organization (IMO) aims to achieve net-zero GHG emissions from international shipping by 2050. Similarly, the aviation sector has proposed halving GHG emissions compared to 2005 levels by 2050. Both sectors prioritize sustainable fuels with maritime investments in alternative fuels like ammonia, methanol, and lignin-alcohol (Li-OH) fuels, and aviation focusing on sustainable aviation fuels (SAFs).

Studies have rarely assessed the environmental impacts of low TRL technologies based on the time required to establish them at full scale. To bridge this gap, we propose a prospective environmental assessment framework integrated shared socioeconomic pathways (SSPs) within a consequential context, providing a realistic picture of the environmental impacts of alternative fuels under varying future scenarios.

Our framework assesses uncertainties in scaling technologies and future scenarios, helping researchers evaluate how alternative fuels align with sustainability goals and guiding decision-makers in selecting technologies that minimize trade-offs while maximizing benefits. A Python-based tool was developed to integrate OpenLCA with an integrated assessment model (IAM)-based consequential prospective database. This tool enables value chain assessments of alternative fuels across SSPs, RCPs, and their TRL-related timeframes until 2050. The analysis includes midpoint and endpoint results from prospective consequential LCAs, offering a robust system-wide perspective for Safe and Sustainable by Design (SSbD) innovations.

An interactive dashboard allows users to explore impacts and damage categories across value chains, SSPs, RCPs, timeframes, and technical options. To demonstrate its applicability, the framework was used to assess the production of SAF via the Fischer Tropsch (FT) process, alongside e-gasoline as a by-product and Li-OH via solvolysis process. These findings illustrate the framework's potential to guide sustainable design and development aligned with SSbD principles, enabling a forward-looking approach to decarbonization in maritime and aviation sectors. This versatile tool fosters safer, more sustainable innovations for a carbon-neutral future.

Disclaimer/Disclosure: This research is supported by two key projects. The MAT-Fuel project (<https://projekter.au.dk/mat-fuels>) is funded by Aktieselskabet D/S Orient's Fond, providing resources to advance the study of sustainable marine fuels. The views and opinions expressed in this abstract are solely those of the authors and do not necessarily reflect the official policies or positions of the funding bodies.

5.03.P-Mo447 Toward Circular Shifting of Phosphate Mining Industry in Morocco: Scenarios and Prospective LCA

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With the global population continuing to grow, the demand for food, particularly processed foods and animal products, has surged, making food security an increasingly pressing issue. Phosphorus (P) plays a critical role in ensuring a stable food supply for the future. However, recent studies reveal that nearly 50% of phosphorus is lost during the mining stage, significantly more than in other life cycle stages.

This work addresses the question: What is the impact of closing the loop on phosphate mining in Morocco? As the world's second-largest producer of phosphate rock, Morocco represents a pivotal case study. A previous assessment of the business-as-usual scenario identified key hotspots, forming the basis for this study's exploration of new mitigation strategies grounded in circularity principles.

Preliminary results offer insights into the projected impacts by 2050 for both SSP1 and SSP2, laying the groundwork for a comparative assessment of the developed scenario's long-term effects. This analysis aims to provide the scientific community and policymakers with actionable knowledge to advance sustainability in phosphate resource management.

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5.03.P-Mo448 Evaluating Sustainability: Life Cycle Assessment of Hybrid Biological-Inorganic Systems for Safe Nitrogen Recovery from Wastewater

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Wastewater treatment plants (WWTPs) are essential for safeguarding public health and producing environmentally friendly, reusable water. However, they face challenges related to high energy consumption and greenhouse gas emissions during treatment processes, particularly in the nitrification-denitrification phase, which is critical for nitrogen removal. The primary challenge is the inefficient recovery of valuable resources, such as nitrogen, in current wastewater management practices. This study aims to explore the integration of hybrid biological-inorganic (HBI) systems within WWTPs as a solution for nitrogen recovery and microbial protein (MP) production, thus enhancing sustainability. To address this challenge, the study incorporates HBI systems that utilize hydrogen-oxidizing bacteria (HOB) and external carbon dioxide (CO₂) to recover nitrogen from wastewater. A dynamic life cycle assessment (LCA) aligned with the shared socioeconomic pathways (SSPs) will be conducted to evaluate the environmental sustainability of this integration. The assessment examined two modes of CO₂ supply: amine-based carbon capture (CC) using monoethanolamine (MEA) and direct air capture (DAC) considering both biogenic/atmospheric and fossil sources. The research employs a prospective assessment of background and foreground changes using principles of consequential life cycle assessment (pCLCA), focusing on scenarios up to 2050. This framework helps ensure that sustainability is considered in the early design stages of innovative projects. Results indicate that HBI systems have the potential to advance wastewater treatment by enhancing sustainability through improved global warming potential (GWP) and effective resource scarcity, thus promoting the development of more environmentally friendly wastewater management practices under SSP2. Additionally, the use of the monoethanol amine (MEA) method for carbon supply yielded better outcomes than direct air capture (DAC) due to effective heat recovery. The instability of HBI system integrated with WWTPs can be attributed to the significant environmental emissions associated with the electrolyzer used for hydrogen production. These findings underscore the need for further optimization of the HBI system to enhance its applicability in various scenarios.

5.04 Advancements in Life-Cycle Inventory (LCI): Enhancing Data Collection and Management, and Addressing Temporal Aspects

5.04.T-01 Supporting the Design of Renewable Energy Scenarios: EnergyPLAN-LCA Framework

Lasse Krogh Poulsen, Iva Ridjan Skov, Henrik Lund, Lotte Ansgaard Thomsen and Soren Lokke, Aalborg University, Denmark

As energy systems are transitioning towards renewable energy in place of fuel combustion, the main environmental impacts are also shifting from direct combustion impacts to indirect construction and maintenance impacts. These types of impacts are rarely accounted for in energy systems modelling (ESM), and therefore, LCA integration in ESM is vital to assess these indirect impacts and potential trade-offs with other environmental impact categories e.g., land use. By integrating prospective LCA and the EnergyPLAN modelling tool, we can simulate different energy transition scenarios which can be assessed at temporal resolutions ranging from a whole year down to a specific hour, including seasonal weather patterns and energy demand fluctuations. These different scenarios can then be compared to account for potential trade-offs and the temporal differences in energy capacity construction versus avoided impacts from electricity production in the longer term. To this end, we use Premise to create prospective background databases, and national energy scenarios to provide foreground systems with relevance for national decision-making.

We have demonstrated the tool on the IDA Climate Response scenarios for a climate-neutral Danish energy system in 2045. It has been found that even though the scenario is climate-neutral when only accounting for combustion emissions, it is not so when accounting for the whole life cycle. However, compared to the reference scenario, it still performs better in the long-term, and the massive renewable energy capacity construction required to realize the IDA scenario pays itself back within 11 years in terms of GHG emissions saved compared to the reference scenario, even when applying GWP100 discounting to future (avoided) emissions. This return on investment can be analyzed across various impact categories.

The relevance of the tool was demonstrated on the IDA scenarios ex-post but can and should ideally be applied in an iterative modelling process between ESM and LCA to ensure modelling of scenarios with minimal environmental impacts on as many impact categories as possible.

5.04.T-02 SHRECC: Simple Hourly-Resolution Electricity Consumption Calculation

Thomas Gibon, Enrico Benetto and Sabina Bednářová, Luxembourg Institute of Science and Technology (LIST), Luxembourg

Rapid changes in electricity supply patterns caused by a growing share of renewable sources and increased international exchanges - can lead to misrepresentations in life cycle inventories (LCI) of electricity mixes over time, resulting in flawed life cycle impact assessment (LCIA) results, particularly for electricity-intensive systems, and incompatibility with new directives.

Databases like ecoinvent, widely used by LCA practitioners, often rely on annual averages from [year n-3] and fail to account for differences in electricity production mix throughout the year. With the rise of intermittent electricity production, production mixes can vary significantly by hour, month, or season. Coupled with fluctuating consumption, relying on average mixes can distort life cycle impact estimates. This challenges the notion that electricity mixes variability reflects ontic uncertainty, as we argue it can be systematically captured to enhance result reliability, reduce uncertainty, and potentially optimize studied systems.

This study addresses this research gap by developing a tool (a python package shrecc) that collects high-resolution hourly electricity high voltage production and consumption data via the Energy Charts API. The data is parsed, matched with ecoinvent classifications using ENTSO-E datasets, converted to low-voltage mixes, and made available as a ready-to-use brightway-compatible database. Practitioners can select countries and time intervals (one-off or reoccurring), enabling more precise and user-friendly integration into LCA models.

We show the impact of our tool on an LCA study of an electric agricultural robot. The functional unit is manufacture and operation of one electric robot for its lifespan of 15 years in France and Greece. The robot must be charged before its operation for 1h/day, and such charging can take place at different times of the day. We used brightway for the LCA and the shrecc package to produce low-voltage electricity mix LCIs used in the operation phase. We showcase the importance of selecting the charging hour versus using the average electricity mix, on two different electricity mixes: carbon intensive and low carbon mix. Selecting a specific hour (8am and 10pm) compared to using the annual mix shows systematically lower LCIA scores, in both countries and all 6 selected impact categories. This highlights the importance of using time-specific electricity LCIs in LCA studies.

5.04.T-03 Toxicity Assessment of Agricultural Raw Materials: Addressing the Major Shortcoming of Substances' Inventory Data in a Changing (Regulatory, Technological and Climatic) Landscape

Joséphine Gatin and Anne Asselin, SAYARI, France

The assessment of toxicity impacts from chemical substances in Life Cycle Assessment (LCA) faces challenges due to data gaps in Life Cycle Impact Assessment (LCIA) and Life Cycle Inventory (LCI). Notably, there is a lack of consistent, specific and up-to-date inventories regarding types and quantities of plant protection products (PPPs) used for various agricultural productions. Our research addresses this data gap. We developed a model and tool to identify the PPP active substances names and quantities used for major French crops productions. It relies on 3 complementary national public databases:

- The Farm Accountancy Data Network (FADN), providing annual regional agricultural productions and economic PPP purchase data
- The National Phytopharmaceutical Sales Database (BNV-d), detailing annual French PPP purchase volumes by region
- The Ephy regulatory database, specifying active substances contained in PPP and their authorized uses on French agricultural productions.

Combination of those 3 databases enabled us to generate active substances inventories for 51 French productions, with granularity at active substance level. The inventories were generated for each year between 2015 and 2022 at both regional and national scales. The results were compared to the PPP inventories of Agribalyse 3.0. showing consistency with our results. We then assessed the representation of these active substances in commonly used impact assessment methods. A substantial proportion of the substances in our inventories were not characterized in USEtox2.13 (ecotoxicity). In contrast, the Environmental Footprint (EF3.1) method demonstrated significantly better coverage of active substances; however, it still did not account for all substances identified in our inventories. Our method allowed for the automated generation of detailed substances' inventories for major French agricultural productions, using yearly updated databases to reflect changes in regulations and usage. This approach enhances the

granularity of PPP inventories, facilitating their integration with increasingly precise toxicity assessment methods. However, further validation of the model is necessary (and on-going), particularly through comparisons with additional data sources, to ensure its robustness and reliability.

5.04.T-04 Inventory Data Curing for Circular Clothing Business Models and Uncertainty

Sarah Gray¹, Jhuma Sadhukhan², Angela Druckman² and Keith James¹, (1)*The Waste and Resources Action Programme (WRAP), United Kingdom, (2)University of Surrey, United Kingdom*

This presentation will outline steps taken to improve the quality of data available for decision-making about circular clothing business models (CCBMs), such as sale or rental of secondhand clothing. Including product use in life cycle assessment (LCA) is valuable where the use phase is important to the results. For CCBMs, including use is essential to reflect the difference that a change in business model makes to the lifespan of the garment.

A review of existing life cycle inventory (LCI) data was conducted for clothing use and CCBMs and data compiled to represent both conventional clothing acquisition and use and that using a range of different business models. Data in the LCI were quality assessed and each input scored using a Pedigree Matrix approach. Consumer survey data for the use phase of clothing was accessed to improve on the LCI data for critical aspects including the number of garments in the wardrobe and duration of use. An LCA was carried out, including analysis of a variety of scenarios followed by uncertainty analysis using Monte Carlo simulation.

The results of the review of LCI data showed scope to improve available data concerning garment use, especially concerning garments acquired using CCBMs. The results of the LCA showed significant reduction in environmental impacts could be achieved by acquiring half of garments in the average UK wardrobe with the CCBMs. There was variation in the level of reduction between CCBMs and between environmental impact indicators. Online resale showed the greatest reduction but the reduction was not significantly greater than other resale business models. Displacement of new product sales was particularly important to the results.

Following a review, it was possible to improve upon previously existing data for clothing use, particularly clothing acquired using CCBMs in the UK. It was confirmed that CCBMs reduce the environmental impact of clothing relative to conventional, linear extraction, production and disposal of clothing. The results achieved depend upon a number of different factors and importantly include the ability of CCBMs to replace a proportion of linear sales.

5.04.P-Tu407 Methodological Updates in the Agricultural Sector in the Ecoinvent Database

Francesco Cirone, Andreas Giakoumatos and Simone Fazio, *ecoinvent, Switzerland*

Ecoinvent is a comprehensive LCI database that provides reliable and transparent information on the environmental impacts of products and services. Recently several changes have been made in format, content, LCIA methods, and sector-specific methodological improvements. Within them, relevant updates are implemented for the 'Agriculture, Fishery and Animal Husbandry' sector to increase transparency and precision of data. The methodological changes need to overcome the following issues:

- Use of fuels in Agriculture: Fuels were previously modelled together with the whole agricultural operations, with a pre-defined fuel consumption per hectare. When the consumption declared by the data provider was different, the whole operation was upscaled or downscaled accordingly. In the new approach, the fuel is inventoried separately, but always linked to the specific operation, which will be kept as a fixed amount of one or multiple (in case of multiple passes) hectare(s)
- Remodelling of Pesticide emissions: Emissions were previously reported as 100% emissions to soil. The new approach adopts Pest-LCI model to estimate emissions in different compartments (soil, air, and water). The emissions to crop have not been modelled, since the crop compartment belongs to Technosphere, and the related emissions are allocated to soil (in line with PEF or SALCA)
- Split of Multi-nutrient fertilizers (MNFs): MNFs were modelled as whole fertilizer, leading to potential partial double counting or underestimation for some of the single nutrients. The new approach adopts a pre-modelled split of the single nutrient contribution, also including a specific split of emissions in the production phase, where applicable. It includes a recalculation of the market(s) for fertilisers, where used, to rebalance shares of different nutrients where specific products are considered in more than one market and the quantities (e.g. N and P₂O₅ from ammonium phosphate) are not properly balanced according to the quantity of nutrients' supply from different components.

The applied changes lead to changes in LCIA results for fuels and multi-nutrient fertilizer (the latter a bit more variable), the overall score changes due to pesticide remodelling are also low, but with a different distribution of toxicities according to the different compartments.

5.04.P Advancements in Life-Cycle Inventory (LCI): Enhancing Data Collection and Management, and Addressing Temporal Aspects

5.04.P-Tu401 From Aggregated Data to Urban Building Energy Models to Assess Priorities in Buildings Refurbishment Actions

Antonino Marvuglia and Mohamed Laib, Luxembourg Institute of Science and Technology (LIST), Luxembourg

To help setting up priority actions for renovation at city level, comprehensive building energy models are and will be increasingly needed. Building energy modelling can be divided into simulation (engineering), data-driven and hybrid approaches. Simulation models allow high prediction accuracy but require detailed data about the building (geometry, materials, renovation state, occupancy, etc.). Moreover, they are time-consuming and computation resources intensive, especially when the objective is assessing a large set of buildings. To circumvent these shortcomings, many studies use data-driven surrogate models for energy use prediction in large-scale renovation and design projects. These models require fewer details at the building level because they rely on a simplified representation of the buildings, but necessitate large data sets.

In our study, which is related to the city of Esch sur Alzette (south of Luxembourg), we started from aggregated gas consumption data available at the district level (including only residential buildings) and, by using building-related data and information (year of construction, building archetype, building volume and heated surface, estimated occupancy, and for some buildings also roof and walls U-values and type of windows and boiler system installed) as weighting factors, we used mathematical optimization to allocate the aggregated consumption to the building level. The optimization was performed using the `scipy.optimize.minimize` function of the SciPy Python library, and the objective function to minimize was the difference between the measured aggregated gas consumption data (district scale) and the sum of the allocated (disaggregated) building consumption at building scale. This allowed to create a training and a test set used to train an extreme learning machine (which is a fast and efficient single-layer feedforward neural network) that we then used as surrogate model to predict the expected energy use intensity (i.e. energy used by m² of building heated area) and consequent emission savings of each building under scenarios of future renovation that we designed in previous work conducted on the same city. An estimation of the renovation costs was obtained using a simplified cost calculation procedure relying on cost data published by previous studies, that yielded actualized costs ranging from about 200 /m² to above 1000 /m² depending on the type of renovation simulated.

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5.04.P-Tu402 Evaluation of the Environmental Aspects of Running a Wind-PV Hybrid Park in Terms of Temporal Resolution of Energy Supply and Grid Connection Capacity

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Renewable energy integration is crucial for decarbonizing the energy sector. This study examines the annual utilization of a photovoltaic (PV) and wind hybrid park in Schattendorf, Austria. During episodes of simultaneous high wind and solar availability, the existing grid connection capacity limits grid feed-in and currently forces curtailments of the wind turbine or PV output. This results in a loss of renewable energy of approximately 20 MWh or 0,1 % of the total wind output of this hybrid park. This study will assess several scenarios with the aim to environmentally optimize the temporal management of hybrid parks. Two key questions are addressed:

1. By how much can environmental impacts be reduced by using a storage system that shifts the surplus electricity to hours with a higher proportion of fossil fuels in the grid mix?
2. What ecological benefits can be gained when the excess energy is instead fully integrated into the Austrian grid by extending the grid connection capacity?

The core challenge lies in balancing energy supply with the grid connection capacity without sacrificing potential renewable output. For the assessment LCI-data in a similar temporal resolution is necessary. So our approach combines empirical data from on-site measurements, calculated performance metrics, and publicly available data on energy mixes to analyze the extent of energy loss and explore mitigation strategies.

Methodologically, the study employs data analysis, scenario modeling, and storage simulations to determine the optimal conditions for enhanced energy storage and utilization. Data will be evaluated with an hourly resolution.

Preliminary results suggest a substantial reduction potential for climate change impacts if curtailments could be minimized or avoided. Results will compare the environmental impacts of various utilization scenarios, analyze the contributions of various system components and of the turbine lifetime. In a sensitivity analysis, the effect of battery size will be explored and simulations will be conducted to determine the optimal storage capacity.

The findings contribute to advancing flexible supply and demand solutions within the energy sector, offering practical insights into how hybrid parks can optimize renewable energy contributions under grid constraints. This research provides valuable case study data for policymakers and energy planners aiming to enhance the resilience and sustainability by better integrating hybrid renewable sources.

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5.04.P-Tu403 Challenges on the Upscaling of Self-Assembled Monolayer Used in Perovskite PV from a LCA Perspective

Afzal khan Peerukhan, Roberto Magnifico and Angelique Leonard, University of Liège, Belgium

In the last decade, Self-Assembled Monolayers have become highly effective interfacial layers for perovskite PV devices because of their prime role in enhancing device efficiency, stability, and charge extraction. SAMs are single-molecule-thick films that spontaneously organize on surfaces through molecular self-assembly, offering a precise way to tune surface chemistry and energy alignment.

Among various SAM materials, MeO-2PACZ and MeO-4PACZ are the two compounds that have demonstrated significant potential for use in perovskite PV. Despite these advancements, there are significant challenges to the upscale of SAMs data, particularly from a life cycle assessment (LCA) perspective. On a lab scale, the synthesis of SAM molecules often involves complex, multi-step organic chemistry that requires high-purity reagents and large volumes of solvents and energy consumption leading to increased environmental impact. Laboratory conditions are not optimized for better materials and energy efficiency, making it difficult to scale up parameters like energy use and emissions accurately. The upscaling process introduces uncertainties and requires careful assumptions about process efficiencies and supply chain factors. Despite these challenges, upscaling is essential to mimic real-world industrial settings and ensure that LCA results reflect the true impacts of large-scale production.

In short, key challenges for the upscaling of LCI data, are inadequate inventory data and data quality, material input, energy consumption, process yield, and finally waste generation and management at end of life. This study provides a comprehensive comparison of the lab and industrial scale LCA of MeO-2PACZ and MeO-4PACZ used in perovskite PVs. It examines the LCA implications of upscaling of MeO-2PACZ and MeO-4PACZ production and highlights environmental Impacts. This study also provides a correlation between lab-scale and industrial scale data, facilitating upscaling efforts of similar chemicals when industrial data is not available. Primary data have been gathered from project partners, supplemented by secondary data from Ecoinvent v3.9.1. The LCA study is done according to the ISO 14040/44 standards.

5.04.P-Tu404 Using Geography in LCI collection: An LCA Model to Assess the European Wind Turbine Fleet

Dominik Huber and Maeva Lavigne Philippot, Vrije Universiteit Brussel, Belgium

Various LCA models exist for the evaluation of wind power, all taking into account, to some extent, spatially refined LCI data. A model capable of evaluating the environmental impact of potential wind turbines in Europe is currently lacking, due to this large scope coming along with extensive data. Thus, we present a new LCA model that considers turbines location all over Europe scalable to its rated power to produce spatially differentiated results.

We build on WIND_LCA_DK, an open-source model developed to assess the Danish wind turbine fleet and further develop it to assess wind turbines on the European continent. While the model is capable of scaling the LCI to the rated power, in the case study, 3.4MW onshore turbines are investigated for installations in France, Italy, Norway, and Germany. First, the location (longitude/latitude) is added as an argument to determine the LCI data of a single turbine. The location data is then used to obtain the following LCI data: a) the type of foundation (offshore/onshore), b) the type of foundation including their material for different foundation types based on the sea depth, c) the produced electricity of the turbine and d) the ISO country code to identify relevant ecoinvent processes, cable length for grid connection and

transportation distances.

The French wind turbine has, with 24 gCO₂eq/kWh, the highest climate change impact (CC), whereas the other turbines result in between 17 and 18 gCO₂eq/kWh. As the rated power of all wind turbines is 3.4MW, the French turbine yields the lowest production and the CC of the French electricity mix is low, the CC difference can be attributed to adjusted cable length or transportation distances.

The LCI collection is enhanced by data from geographical sources, e.g., transportation distance, connection cable length and sea depth. The modelled case study results in different environmental performances depending on their location, which can be linked to improved LCI collection.

5.04.P-Tu405 Powering the Future: Integrating Battery Degradation into Battery Life Cycle Assessment

Zhi Cao, Xin Chen and Chunli Chu, Nankai University, China (Mainland)

Achieving a sustainable low-carbon transition via electric vehicles hinges on a thorough understanding of the carbon footprint of lithium-ion batteries, especially during their use phase. This aspect is often neglected due to the varied battery application scenarios and the inherent uncertainty and complexity associated with battery service life. Factors such as battery efficiency and degradation are crucial in determining the indirect greenhouse gas (GHG) emissions during this phase. Therefore, integrating a battery degradation model into the use phase assessment is vital for a more precise evaluation of the carbon footprint.

In this project, we develop a comprehensive model that integrates the battery lifetime model within a life cycle assessment (LCA) framework. This model will estimate both cradle-to-gate and use phase GHG emissions for five battery chemistries: LFP, NCA, LMO, NMC111, and NMC811. We aim to examine how regional differences in user charging habits and ambient temperatures affect battery durability and subsequently influence GHG emissions. Moreover, we will quantify the impacts of battery efficiency and user charging habits on the GHG emissions across different life stages of the battery, employing sensitivity analysis to deepen our understanding of the relationship between battery performance, usage patterns, and GHG emissions.

We aim to address the following research questions: (1) What are the GHG emissions of different battery chemistries in relation to their durability? (2) How does battery efficiency impact GHG emissions during the use phase? (3) How do variations in battery durability, influenced by different usage scenarios, affect both cradle-to-gate and use phase GHG emissions? This project provides detailed insights into the life cycle emissions of batteries and offers guidance on optimizing battery usage to minimize GHG emissions.

5.04.P-Tu406 A Novel Tool for Generating Hybrid LCA Databases

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We present a novel open-source tool for generating hybrid life-cycle assessment databases. To showcase the utility of this software, we present LCA results based on two hybrid databases generated by combining the USEEIO/USLCI and Exiobase/Ecoinvent databases. We show that the environmental impact of production processes ("activities") changes significantly through hybridization - as additional inputs from the input-output sectors are added through upstream connections. Changes are up to 100% for multiple impact categories.

While hybrid life-cycle assessment has long been known to improve the accuracy of environmental impact assessment, a lack of tools for hybridizing databases has been cited by all recent reviews as a key reason hampering the adoption of the method by practitioners. A first open-source package named "pyLCAIO" by Agez (Polytechnique Montreal) was first released in 2020, but this tool was intended to hybridize specific versions of specific databases only. In contrast, our tool allows practitioners a maximum amount of flexibility - with regards to databases, input data and output data.

To our knowledge, this is the first time, hybridization of multiple databases has been made possible to users. We are confident that our tool and our hybridization results will be interesting to a broad audience - both LCA practitioners and sustainability researchers more broadly.

5.04.P-Tu407 Methodological Updates in the Agricultural Sector in the Ecoinvent Database

Francesco Cirone, Andreas Giakoumatos and Simone Fazio, ecoinvent, Switzerland

Ecoinvent is a comprehensive LCI database that provides reliable and transparent information on the

environmental impacts of products and services. Recently several changes have been made in format, content, LCIA methods, and sector-specific methodological improvements. Within them, relevant updates are implemented for the 'Agriculture, Fishery and Animal Husbandry' sector to increase transparency and precision of data. The methodological changes need to overcome the following issues:

- Use of fuels in Agriculture: Fuels were previously modelled together with the whole agricultural operations, with a pre-defined fuel consumption per hectare. When the consumption declared by the data provider was different, the whole operation was upscaled or downscaled accordingly. In the new approach, the fuel is inventoried separately, but always linked to the specific operation, which will be kept as a fixed amount of one or multiple (in case of multiple passes) hectare(s)
- Remodelling of Pesticide emissions: Emissions were previously reported as 100% emissions to soil. The new approach adopts Pest-LCI model to estimate emissions in different compartments (soil, air, and water). The emissions to crop have not been modelled, since the crop compartment belongs to Technosphere, and the related emissions are allocated to soil (in line with PEF or SALCA)
- Split of Multi-nutrient fertilizers (MNFs): MNFs were modelled as whole fertilizer, leading to potential partial double counting or underestimation for some of the single nutrients. The new approach adopts a pre-modelled split of the single nutrient contribution, also including a specific split of emissions in the production phase, where applicable. It includes a recalculation of the market(s) for fertilisers, where used, to rebalance shares of different nutrients where specific products are considered in more than one market and the quantities (e.g. N and P₂O₅ from ammonium phosphate) are not properly balanced according to the quantity of nutrients' supply from different components.

The applied changes lead to changes in LCIA results for fuels and multi-nutrient fertilizer (the latter a bit more variable), the overall score changes due to pesticide remodelling are also low, but with a different distribution of toxicities according to the different compartments.

5.04.P-Tu408 Identifying Key Predictors for Biodiversity, Water and Climate Footprints of Crop Production

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Crop production is one of the key drivers of greenhouse gas (GHG) emissions, land and water use globally. Life cycle assessment of these (and other) environmental impacts for crop-location-management practice combinations is highly relevant for understanding the magnitude of impacts and for exploring potential mitigation options. However, primary farm-level data are difficult to gather and secondary datasets (life cycle inventories; LCIs) are missing for many crop species, farming practices and regions. As LCI data gaps are manifold, it is necessary to prioritize data compilation efforts, focusing on the inventory flows that contribute most to environmental impacts. Here, we identify the most important LCI input flows, up to the farm gate, in terms of their contributions to biodiversity loss, water use and GHG emissions. To that end, we model these three environmental footprints for 92 crops across 44 regions based on Ecoinvent and Agribalyse LCI data and using the ReCiPe2016 impact assessment methodology. Subsequently, we apply five modelling techniques (Random Forest, K-Nearest Neighbors, Artificial Neural Network, Generalized Boosting Method, and Linear Modeling) to predict the life cycle footprints based on 27 groups of farming inputs, including fertilizer application, pesticide use, tillage, and yield. We find that the Generalized Boosting Method outperforms other modelling techniques when using all 27 predictors, achieving a cross-validated explained variance of 84-88% for the three environmental footprints. Random Forest performs best when lowering the number of predictors to less than ten. A Random Forest model with seven predictors achieves a cross-validated explained variance of 81-88%. Key predictors of biodiversity footprints are yield and fertilizer input. For water footprints, irrigation, fuel input, and pesticide input are the most important. GHG footprints are mostly related to yield, fuel, heat, electricity and fertilizer input. Our study offers a systematic approach to identify key predictors of crop production impacts, facilitating targeted crop LCI gap-filling.

5.04.P-Tu410 Ensuring Environmental Integrity: A Critical Assessment of Mass Balance in Food Packaging LCA

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The mass balance approach is a chain of custody model used in a variety of industries that allows for the mixing of sustainable and non-sustainable materials. This approach is based on established standards and auditable bookkeeping. However, the mass balance approach has some limitations. The problem with mass balance is that it does not reflect the physical properties of the material or ensure physical traceability. Nevertheless, it is not suitable for all types of materials. For example, it cannot be used for complex materials such as plastic waste, which contains a mixture of different polymers. In these cases, other methods, such as carbon counting or net calorific value, must be used to determine the chemical

value of the recycled feedstock. In mass balance the calculations are not based on the physical relationship between the input resources and final product content, meaning e.g. that a product might be sold as biobased but still contains only 5 % biobased materials. Even though, there are existing mass balance certification systems and mass balanced products already in the markets, there is no established and regulated market for products attributed materials, and ensuring no double-counting or green washing exists is difficult. Additionally, the LCA standards and PEF do not address the mass balance approach, and there are no clear guidelines on how to use mass balance in LCA. This makes it difficult to assess the environmental impact of products that use the mass balance approach. In this case, how to consider mass balance in a national PEF-wise guidance for LCA of food packaging? To address these issues, there is a clear need for a standardized methodology for conducting Food Packaging LCA studies that aligns with the PEF framework. This methodology should provide specific instructions on how to account for the environmental impact of products made from different materials with different attributable properties, coming from various sources. By developing and implementing such a standardized approach, companies can more accurately communicate the environmental performance of their products to consumers.

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5.04.P-Tu411 Transforming LCI Data for Plastic Packaging Using LLMs: A Path Toward Greater Accuracy, Transparency, and Efficiency

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Life Cycle Inventory (LCI) data is essential for Life Cycle Assessment (LCA), particularly in calculating Global Warming Potential (GWP) under the European Union's Product Environmental Footprint (PEF) initiative. By providing consistent and transparent environmental metrics, LCI data helps identify environmental hotspots, supports sustainability assessments, and guides businesses and policymakers toward more sustainable products and processes.

Despite its importance, LCI datasets face significant challenges that limit their reliability and usability. Issues such as data inaccuracies, methodological inconsistencies, and limited documentation hinder the verification and reproducibility of results. Many datasets are also outdated, unable to keep up with technological advances and market dynamics, further compromising their relevance. Moreover, collecting and updating high-quality LCI data is resource-intensive, requiring expertise and significant effort to address regional and sectoral variations and the dynamic nature of industrial processes.

To overcome these challenges, we propose a novel framework that utilizes large language models (LLMs) to automate key aspects of LCI data management, including data extraction, validation, and synthesis. By leveraging LLMs, this approach reduces processing time, minimizes human error, and improves data comprehensiveness. Focusing on plastic packaging one of the most environmentally impactful product categories the framework enhances transparency, timeliness, and granularity. This method addresses critical gaps in LCI data while offering practical insights to advance sustainability practices and meet stringent environmental standards.

5.04.P-Tu412 Advancing Plastic Modelling Methods in Life Cycle Assessment

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Plastics are versatile and complex materials made of chemicals, known as additives, and polymers. The combination of polymers and additives is what makes plastics multi functional across so many applications and industries. Due to the variety in the potential material composition, material flow-analyses (MFA) and life cycle assessments (LCA) of plastics often omit plastics additives from the scope of their study or aggregate them. However, such approaches lead to underestimations of the potential risk cycling, contamination, degradation, and byproducts which result from the behaviour of additives throughout the lifecycles, recycling, and management (or lack of management) of plastics. These oversights may lead to underestimation, generalizations, or omissions of the toxicity potential of plastics both in their linear lifecycle studies and anticipatory circular lifecycle studies.

Moreover, the lack of available data, transparency, and reporting on the quality of and type of additives included in plastics has previously made including additives in MFAs and LCAs difficult, without close shareholder collaboration. Fortunately, researchers across the globe have worked to provide significant insight into the chemicals associated with the production of plastics. In previous research we have mapped

this data to LCA data availability. In this study we have mapped the chemicals associated with the production of plastics to impact methods and characterization factors. We have then created a python package to simulate the chemical and polymer composition of plastics (potential or matched to user input) to available LCA data, allow the user to select close proxies or assemble additive portfolios for unmatched chemicals, and score the completeness and data quality of the flows included in the mixture. These portfolios can be used by the LCA practitioner to create better models of plastics in LCAs and assess which databases provide the best available data for the plastic under study. The package then allows the user to either export the data as an excel to use when modelling in their chosen software or can be linked to Brightway.

This package is then used to model and demonstrate how to include additives in LCAs of plastics in various end of life and waste management pathways for a case study of cosmetic packaging can help to assess the potential additive contamination, degradation rates, virgin additive needs in multiple loop life cycles for plastic materials.

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5.04.P-Tu413 Prospective LCA of Road Transport across Various Powertrain Options

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As the transportation sector aims for rapid transformation toward sustainable mobility, understanding the environmental impacts of various powertrain technologies is crucial. This study presents a prospective life-cycle assessment (LCA) of diverse road transportation options, including cars, light commercial vehicles, heavy-duty trucks, and buses, across various powertrain configurations: internal combustion engine (diesel, petrol, and compressed natural gas), battery electric, plug-in hybrid, and fuel cell electric. Designed to reflect average European conditions, the analysis draws primary data from an extensive literature review and laboratory measurements, ensuring robust and representative inputs for each powertrain and vehicle type. The analysis is further distinguished in leveraging prospective LCA through coupling background databases with projections from Integrated Assessment Models (IAMs), to assess potential future environmental impacts of each individual powertrain option. By incorporating IAM predictions, this LCA not only provides insights into current environmental burdens but also explores their evolution under different technological and policy scenarios. The findings aim to guide stakeholders in making informed decisions on sustainable transportation solutions by highlighting the varying environmental impacts over time of different vehicle and powertrain combinations. This research thus contributes to advancing transportation LCA methodologies and aligns with global efforts to minimize environmental footprints in the mobility sector.

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5.04.P-Tu415 Progress of Carbon Intensity of Fossil Fuels in Brazil

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Several actions have been carried out in the transportation sector to support the energy transition, preparing the current energy systems for a market less dependent on fossil fuels. RenovaBio is a Brazilian program that encourages the increasing introduction of renewable fuels, through a program of carbon credits, acquired by comparison with the fossil fuels they are replacing. For this reason, the objective of this paper is to update the emissions estimate for the production of gasoline and diesel used as a reference in RenovaBio. The development of this work was based on the well-to-wheel life-cycle assessment methodology, focused on carbon intensity (CI). Given that Petrobras is responsible for 88% of oil, 83% of gasoline, and 88% of diesel productions in the country, a "top/down" approach was adopted using its results from public data available in company sustainability reports audited by a third party and statistical sector publications. To calculate the CIs, the imported portions of oil and ready-made fuels were considered, adding the specific carbon emission from each country as well as the from transportation using factors from Ecoinvent 3.9. Refining emissions were estimated by combining the individual production of each of the 10 refineries of the Petrobras group along with simple energy allocation applied for the various co-products. The results obtained show a reduction in carbon intensity of 4% found for both fuels in the period from 2010 to 2023, reaching values of 82.8 and 87.2 gCO₂e/MJ for gasoline and diesel,

respectively. However, when only the E&P, transportation and refining stages are considered, the reduction achieved is 33.5%. This improvement in fossil fuel production efficiency can be partially explained by the high share of oil coming from the sea (97.7%) through modern extraction technologies in deep and ultra-deep waters, which has much smaller environmental impacts than other technologies. Furthermore, Petrobras, which has been complying with its strategic plan to reduce these emissions, has implemented several improvements to reduce the burning of gases in the flare, the energy consumption in refineries, and the losses, among other actions. Comparing the CIs obtained in the present study for the reference year of 2010, Brazilian fuels already had lower emissions of gasoline (14 and 9%) and diesel (10 and 4%) than those of the Low Carbon Fuel Standard of California and the Renewable European Directive, respectively.

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5.04.P-Tu416 A Critical Analysis of EF's Carbon Footprint Formula in the Context of Food Packaging LCA

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There has been long a debate and different practices on interpreting the ISO14044's recommendations on multifunctionality problems in life cycle assessment (LCA) and whether system expansion can be used in attributional LCA (ALCA) or should it be considered consequential LCA (CLCA). Traditionally ALCA has been used for product footprints and CLCA to model impact of marginal changes to guide e.g. decision making. The approaches are mixed in some established assessment methods, e.g. environmental product declarations allow for credits from End-of-Life (EoL) declared as additional information. The European Commission's Environmental Footprint (EF) method is also mixing the approaches as the Circular Footprint Formula (CFF) addressing the EoL modelling has consequential parts. Using different approaches in modelling different parts of the life cycle can be considered illogical. Methods used in LCAs targeted to external communication should be harmonized and consistent, leaving no room for different assumptions.

Recyclability is one key aspect in the sustainability of packaging materials, but difficult to include in strictly attributional approach. Therefore for packaging materials it has been common to use system expansion to avoid allocation or to include credits from avoided burdens from EoL to the packaging material's footprint. This has a significant impact on the results and leads to situations with different assumptions on the substitutions making comparisons difficult. The EF's CFF formula is trying to depict the environmental benefits of recyclability. However, one major consequence of using the CFF formula is the risk of double counting. In case e.g. a packaging material is credited for incineration, this should be considered also in the energy production as usually energy production considers waste as a burden-free input. This means that all environmental impacts related to energy production with waste should be recalculated to avoid double counting. This would be very laborious. Other options could be to use allocation to share the burdens between production systems. This would encourage the use of recycled materials in packaging, but it does not encourage recyclability at EoL. The aim of this study is to find based on literature and case studies the most suitable way to model the EoL of packaging materials in environmental footprints aimed for consumer communication. The aim is to develop a PCR for food packaging aligned with the EF method.

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5.04.P-Tu417 Evaluation of the Carbon Footprint of Ferroalloys used in Stainless Steel Manufacturing

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Ferroalloys are iron-based alloys containing a high proportion of elements such as chromium, nickel, silicon, manganese, etc. They are mainly used by the metallurgical industries, particularly in the stainless steel manufacturing process. The production of ferroalloys is an energy-intensive industrial sector generating significant CO₂ emissions, the main greenhouse gas (GHG). Therefore, it is crucial to evaluate these emissions and understand their origins to reduce the carbon footprint of ferroalloys, contributing to sustainability objectives and the optimisation of energy costs.

Life Cycle Assessment (LCA) is a key method for assessing the carbon footprint of ferroalloys. This study conducted an in-depth exploration of the energy consumption and carbon footprint associated with

ferroalloy production, drawing on various LCA literature sources. The obtained results were then compared to the database of the Gabi software (LCA for Experts 10.8).

The results showed that a set of parameters influences the carbon footprint of ferroalloys and these parameters vary depending on the type of ferroalloy. Therefore, for a downstream consumer, such as a steel manufacturer, it is difficult to directly assess the carbon footprint based only on the quantity of elements in the alloy, especially when the alloy comes from different suppliers located in various countries.

The origin of electricity plays a key role in the environmental impact of all ferroalloys: renewable electricity could significantly reduce CO₂ emissions compared to coal-based electricity. For ferrochrome (FeCr), the type of furnace also has a large influence on the amount of energy consumed. In ferronickel (FeNi), a reduction in CO₂ emissions is possible by comparing the ore type, the Ni content of the ore, as well as the allocation strategy. For ferrosilicon (FeSi), increasing the percentage of silicon (Si) directly affects both energy and non-energy consumption, consequently increasing CO₂ emissions. In ferromanganese (FeMn), higher CO₂ emissions were observed for FeMn with medium carbon content (<1.5%), which is produced from high carbon FeMn. LCAs carried out with the same assumptions for all ferroalloys showed an increase in the carbon footprint in the following order: FeMn < FeCr < FeSi < FeNi. This study highlighted all the key parameters for evaluating the carbon footprint of the mentioned ferroalloys for steel fabricators, all compiled in a single investigation.

5.05 Methodological Advancements in Life Cycle Assessment of Emerging Bio-Based Systems

5.05.T-01 Challenges in Substituting Fossil Products with Bio-based Alternatives

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Bio-based products bring major environmental benefits in terms of improved sustainability, reduced dependence on non-renewable resources, and greater circularity. However, substituting fossil-based products with their bio-based alternatives presents several challenges, given the dissimilarities in material properties, regulatory constraints, and technical performances. These should be taken into account in Life Cycle Assessment studies comparing alternative bio- and fossil-based products. This study explores the challenges related to replacing fossil-based materials with bio-based alternatives in five key sectors: construction, packaging, textiles, chemicals, and woodworking. Using a mixed methods approach consisting of a review of scientific literature and surveys with stakeholders, the research identifies key technical, regulatory, and market-specific barriers to substituting fossil-based products with bio-based ones. The systematic review and analysis of 42 selected peer-reviewed articles reveal common challenges related to mechanical performance, durability, regulatory restrictions, and processing challenges. In the construction sector, for instance, bio-based materials like Cross Laminated Timber require additional treatments to improve fire resistance and durability. In the packaging sector, bio-based packaging faces issues concerning moisture resistance and high costs. In textiles, bio-based fibers show poor processability and durable performance, while in the chemical sector, feedstock availability is seasonal, which complicates substitution. In the woodworking sector, bio-based adhesives face challenges regarding mechanical strength and resistance to water. In addition, regional differences in regulations can further complicate the substitution. Based on these findings, recommendations are provided for carrying out LCA studies of bio-based products taking into account the specific substitutability conditions.

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5.05.T-02 Tipping Points in Polymer Life Cycle Greenhouse Gas Emissions

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For decades polymers have offered a unique combination of low cost and high durability and are the material of choice for a huge range of applications. In the current energy system, such polymers require the combustion of fossil resources to provide process heat and electricity for manufacture and often release emissions from embodied resources at the end of life. Polymers which are biodegradable or based on biological feedstock avoid some of this resource use, but in some cases have greater energy input requirements than their fossil polymer equivalents. This work compares life cycle greenhouse gas emissions of pairs of fossil- and biologically-based polymers under various UK electricity, heat and end-of-life treatment scenarios between 2023 and 2050. As specific emissions from electricity and heat supply reduce over the period, life cycle emissions of all polymers fall. Polylactic Acid was found to already have

lower emissions than polystyrene in some scenarios. Low-density polyethylene was found to have lower emissions than poly(butylene adipate-co-terephthalate) in all cases to 2040. Bio-based high density polyethylene was found to have lower emissions than its fossil equivalent after 2024, and bio-based polyethylene terephthalate after 2043, depending on energy and end-of-life scenarios. Identifying and acting on these tipping points offers a route to significant reductions in the emissions of polymers, and even suggests that polymers with net negative emissions can be manufactured by 2040, offering the possibility of greenhouse gas sequestration through polymers made from agricultural waste.

5.05.T-03 Upscaling Framework for Laboratory-Scale Life Cycle Assessment

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Environmental issues that are discovered during laboratory-scale Life Cycle Assessment (LCA) in a research and development (R&D) project can be drivers of innovation aiming to reduce the environmental hotspots before industrialization. Production in R&D projects is mostly done on laboratory scale on technology readiness level (TRL) 3, with the aim to reach pre-industrial scale production on TRL 6. Most of the existing upscaling frameworks rely on data being available regarding industrial production processes, electricity requirements for the industrial equipment, and minimum and maximum throughput of the machines. To bridge the gap between laboratory scale and industrial scale production, a framework has been developed that focuses on expert participation by estimations and scenario development from technical partners and literature. The proposed upscaling framework consists of four consecutive steps: 1) scenario definition, 2) upscaling mechanisms, 3) estimation of upscaled production and 4) calculation of the potential environmental impact of the upscaled production. The framework includes scenarios for efficiency increase in equipment, increase in production and optimization of yield, as well as a sensitivity analysis. The framework is showcased using the laboratory scale production of bio-based electrodes intended for supercapacitor (SC) application. In the laboratory-scale LCA of the electrode electricity requirements during production represent the largest hotspot, being responsible for 65 % of the total CO₂-eq emission. During laboratory-scale production, the individual additives and solvents have only a small influence on the environmental impact, whereas after upscaling CO₂ and phosphoric acid represent the largest environmental hotspots. In total, the results of the upscaled production processes depict a potential reduction in Global Warming Potential (GWP) of up to 90 % compared to laboratory scale production. All scenarios show the drastic reduction of the environmental impacts when produced on a larger scale and how environmental hotspots are shifted away from electricity requirements towards materials, additive and solvent inputs. The proposed framework can be applied in R&D projects to guide technical experts in resource selection and optimization of production processes. It has proven valuable in anticipating the environmental burden and burden shifting associated with upscaling production.

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5.05.T-04 Job Creation Potential Tool for Assessing Employment Opportunities in a Bio-Economy Context

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Social Life Cycle Assessment (S-LCA) is a methodology employed to assess the social impacts of products and services throughout their life cycle. The UNEP/SETAC Life Cycle Initiative's guidelines on S-LCA, constitute the primary reference in the field. This methodology has a relative low level of mature, apart from presenting several gaps when it is referred to the consideration of addressing the particularities of Bio-based Systems (BbS).

Putting the focus on one of them, it was identified a lack of inclusion of key concepts related to bio-economy, such as employment opportunities. The latter is in relation to one of the European Union s Bioeconomy Strategy, which include the creation of jobs. Therefore, in response to the limited availability of quantitative social indicators, one standardized procedure has been proposed to be followed for the estimation of the Job Creation Potential (JCP).

The JCP is a social indicator that quantifies the total number of jobs that a product system can generate through the supply chain. In this sense, it represents the aggregation of jobs created internally (in-house)

and those created by additional upstream life cycle stages.

The inclusion of the JCP methodology in the S-LCA represents an important methodological advance for conducting S-LCA to BbS. In addition, an easy-to-use online tool in the form of an Excel file is expected to be applied in a series of 10 case studies from five bio-based sectors: (i) construction, (ii) pulp and paper, (iii) textiles, (iv) biochemicals and (v) woodworking. It is also expected that this online tool will be further refined by differentiating the jobs created not only according to their origin (in-house or upstream), but also according to their skill level (high-, medium- and low-skilled work) and gender (male or female). Thanks to the development of a methodology for estimating the potential jobs created, it was possible not only to extend the range of procedures for applying an S-LCA, but also to address some of the social concerns included in the main strategies at EU level for implementing a bioeconomy. Notwithstanding, it was recognized that the inclusion of job losses due to bio-based activities is important to obtain a complete and accurate picture of the employment situation. Consequently, it is important that future research focuses on developing a more complete methodology that accurately captures both job creation and job losses.

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5.05.P-Tu421 Evaluation of the environmental impacts of three novel bio-based alternative solutions through a Life Cycle Assessment (LCA)

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GREEN-LOOP Project aims to transition to a more sustainable manufacturing system by developing a new series of innovative bio-based materials that can replace the common materials used in the market (petroleum-based ones). The project is focused on three different bio-value chains, demonstrating the potential of these new solutions to detect critical industrial needs and decrease their environmental impacts. During the first value chain (VC), the development of a bio-rubber with lignin additives from the paper industry will be studied. The use case of this VC is a multifunctional panel with new properties, such as the enhancement in flame retardant, or vibrating-damping properties for the construction sector. The use of biodegradable bioplastics in bottle closures is the use case for the second VC. Inside the food and beverage sector, the objectives of these closures are the olive oil and limoncello bottles. Finally, the last VC will assess wood composites for application in different industrial sectors. The composites are made with bio-based materials and aim to substitute the conventional material in the manufacturing of components, as well as sliding bearings. One of the main motivations for GREEN-LOOP Project is to improve the mechanical, physical, and chemical properties of bio-based products to make them viable in comparison to traditional composites, ensuring a lower environmental impact. A LCA is carried out to evaluate the sustainability of the GREEN-LOOP's development. The LCA is focused on environmental impacts from the beginning of the value chain (raw materials extraction), considering the recyclability or the waste reduction. The main project allegations say that the bio-based solutions have competitive mechanical performance with a reduction in the environmental footprint. These results talk about the potential for the materials and play an important role in the shift toward more sustainable manufacturing, providing viable solutions for industrial sectors to know environmental laws and the demand from the consumers related to the bioproducts. In summary, GREEN-LOOP Project offers innovative alternatives to traditional materials, while reinforcing the economic benefits of circular economy approaches and ensuring sustainability throughout the product lifecycle. This initiative lays the foundations for the widespread use of bio-based materials in various industries, contributing to a more sustainable and resilient future.

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5.05.P-Tu424 Assessing Environmental Performance Improvements in Microalgae-based Products via the ALIGNED Framework

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The bio-based industry is important in addressing global challenges such as climate change and resource

depletion. This study examines the environmental impact of a multi-product microalgae biorefinery producing omega-3-rich lipids for edible oils, protein for dietary supplements, and a protein-carbohydrate mix for animal feed. The aim of the study is to test and evaluate how the ALIGNED framework for the life cycle assessment (LCA) of bio-based products can be used in practice to assess and improve the environmental performance of microalgae cultivation and downstream processes through identifying and implementing alternatives with lower impact. The learnings shall be applicable beyond the single case to the broader microalgae aquaculture sector. Several methods and tools developed in the ALIGNED project framework were implemented, in particular the methods for prospective background modelling (LCI), the methods for biogenic carbon accounting (LCIA) and for uncertainty analysis (interpretation), and methods for data structure and input (LCI), among others. The study evaluates a base scenario alongside alternative scenarios, such as the integration of on-site photovoltaic energy supply. Prospective analyses are conducted to explore future scenarios using predictive models. This is especially relevant as the case study represents an emerging industry. Results of the specific case considered show that the cultivation process has the largest environmental impact compared to harvesting and biorefinery, electricity being the main contributor to this impact, along with carbon dioxide and nitrogen source supply. The ALIGNED framework has provided tools accessible to non-experts, although further work can still be done to enhance the accessibility and simplify the experience.

5.05.P-Tu426 Towards the Integration of Bioeconomy Constraints in Life Cycle Assessment

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The transition to an EU-level bioeconomy will induce a growing demand for biomass feedstocks. The extent to which the production of different biomass feedstock types can respond to the increased demand is limited by many factors. This leads to competition between sectors demanding the same, finite biomass feedstocks. Considering these constraints and competition when conducting a Life Cycle Assessment (LCA) of bio-based technologies is fundamental. We developed a framework that allows to construct marginal market mixes for bio-based products and feedstocks by adapting EU-wide data on biomass production and use for applications in LCA.

We use an EU JRC dataset on biomass production, supply, uses, and flows. The dataset allows for a comprehensive cross-sectoral representation of the EU bioeconomy. It provides detailed biomass flows linked via supply and use data across different sectors. The cross-sectoral mass-based data is used to investigate seven bioeconomy sectors and capture the indirect effects of the corresponding fossil sectors on their markets.

Through consequential LCA, using JRC-derived economic data and policy trends, we forecast the future development of the supply and identify the unconstrained production activities, those with the capacity to increase production that will respond to the increased demand. We then create new market activities that represent the projected share of these activities in the marginal mix for biomass feedstocks.

We represent the seven targeted sectors and the relevant marginal mixes in a matrix format which enables the integration of the proposed model with LCI databases, allowing to capture the competition for biomass between different bio- and non-bio sectors in LCA.

The framework allows to obtain more holistic assessment of the environmental impacts linked with the shift to bioeconomy by computing new marginal mixes that capture the indirect burdens of competing bio-based and fossil sectors. Further work will focus on expanding the framework beyond bio-based products.

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5.05.P Methodological Advancements in Life Cycle Assessment of Emerging Bio-Based Systems

5.05.P-Tu418 More Than Just Business: The Bioeconomy Supporting the Development of New Sustainable Business Models in Bulgaria

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Climate change and its inevitable cataclysms are no longer abstract theories. Just a few days ago, we said goodbye to the summer of 2024, which turned out to be the hottest in Europe since 1950. Temperatures were recorded 1.54°C above the seasonal average. Natural disasters affecting Bulgaria have become commonplace, and while the process cannot be stopped, it can be slowed down. This requires innovative solutions that can be integrated into the economy while also ensuring environmental and social protection. This study analyzes how the bioeconomy in Bulgaria stimulates the emergence of innovative business models based on the principles of sustainable development. Through an in-depth analysis of existing

initiatives and policies, the factors that facilitate or hinder the development of such models are identified. The study also analyzes the link between the bioeconomy and social innovation, examining examples of successful Bulgarian companies that have integrated sustainability into their business practices. In addition, the study will analyze the future prospects for the development of the bioeconomy in Bulgaria, the possible scenarios until 2030 and the barriers facing the business. The research will draw on surveys research and case study analysis of best practices and challenges.

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Environmental sustainability and circularity criteria for industrial bio-based systems

5.05.P-Tu419 30 Years of Life Cycle Assessment (LCA) in Forestry: State of the Art and Methodological Proposal for the Life Cycle Assessment of Forest Operations

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Life cycle assessment (LCA) has been an important tool for assessing the environmental impact of various processes, products and industries since the late 1960s. In the early 1990s, LCA studies were started in the forestry sector, but these analyses lacked consistency and comparability. In 2015, a study by Klein et al. provided a detailed descriptive and quantitative analysis of existing LCA studies focusing on forest operations, with an emphasis on global warming potential (GWP). This work was the cornerstone for our study as it addressed issues such as study objectives, system boundaries, functional units, impact categories and processes involved. A decade later, our study aimed to assess progress in LCA of forestry production from 2013 to 2023 by analyzing recent LCA studies, comparing them with previous research, and updating methodological recommendations for conducting LCA in the forestry sector. Further objectives were to assess progress and remaining challenges in LCA of forest production over the last decade and to highlight the implications of recent LCA studies for policy decisions and sustainable forest management practices.

We analyzed 90 different peer-reviewed studies. The studies showed large differences between the methodological assumptions and the resulting outcomes. Most of the studies analyzed followed a cradle-to-gate approach, where two different system boundaries were identified: from forest to forest road and from forest to plant gate. Most studies investigated wood for energy purposes or as raw material for sawmills. We found many different functional units, expressed by dimension, by area and/or time, by mass or by energy content. A total of 14 impact categories were identified in our study, with global warming being the most frequently studied category. In half of all studies, allocation methods were not mentioned at all. In more than half of the studies, no methodological approach other than the IPCC Guidelines and the EN ISO 14040 and EN ISO 14044 standards was mentioned. If a database was used, ecoinvent was mentioned in most cases.

To enable better comparisons between LCA studies in the forest sector in the future, we propose some methodological approaches to harmonize system boundaries, functional units, considered processes and allocation assumptions or at least a more detailed metadata documentation. These proposals could help to clarify the description of forestry production in existing declarations and standards.

5.05.P-Tu420 Comparative Lifecycle Study of Wooden and Concrete Buildings in Finland: Advancing Holistic Sustainability Assessments

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The construction sector plays a crucial role in shaping global sustainability, contributing to both environmental challenges and positive impacts such as job creation and community well-being. While discussions around sustainable construction often focus on environmental impacts particularly carbon footprint a more comprehensive approach must also consider other key dimensions of sustainability. Therefore, this study examines the sustainability trade-offs and potential benefits of two identical multistorey buildings in Finland, one primarily constructed from wood and the other from concrete, using a comparative lifecycle assessment (LCA) approach that integrates both environmental and socioeconomic considerations.

The environmental LCA assesses impacts beyond carbon emissions across each lifecycle stage, from raw material extraction to end-of-life. To integrate socioeconomic considerations, a Social-LCA evaluates factors such as job creation, community benefits, and wellbeing of building users. This integrated approach facilitates a broader sustainability assessment, demonstrating that materials such as wood can offer notable socioeconomic benefits while also providing environmental advantages, alongside the trade-

offs involved in material selection.

Preliminary results suggest that the wooden building may have a lower environmental footprint, particularly due to reduced fossil energy demands. Socioeconomically, wood construction shows potential for supporting local job markets and rural economic stability. However, both materials present distinct advantages and challenges, highlighting the importance of comprehensive assessments. By addressing these interconnected impacts, this study advances tools and methods for more comprehensive lifecycle assessments of bio-based systems, contributing to the refinement of sustainability frameworks. These insights are particularly relevant for policymakers and industry stakeholders navigating material choices to achieve decarbonization and broader sustainability goals.

5.05.P-Tu421 Evaluation of the Environmental Impacts of Three Novel Bio-Based Alternative Solutions through a Life Cycle Assessment (LCA)

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GREEN-LOOP Project aims to transition to a more sustainable manufacturing system by developing a new series of innovative bio-based materials that can replace the common materials used in the market (petroleum-based ones). The project is focused on three different bio-value chains, demonstrating the potential of these new solutions to detect critical industrial needs and decrease their environmental impacts. During the first value chain (VC), the development of a bio-rubber with lignin additives from the paper industry will be studied. The use case of this VC is a multifunctional panel with new properties, such as the enhancement in flame retardant, or vibrating-dumping properties for the construction sector. The use of biodegradable bioplastics in bottle closures is the use case for the second VC. Inside the food and beverage sector, the objectives of these closures are the olive oil and limoncello bottles. Finally, the last VC will assess wood composites for application in different industrial sectors. The composites are made with bio-based materials and aim to substitute the conventional material in the manufacturing of components, as well as sliding bearings. One of the main motivations for GREEN-LOOP Project is to improve the mechanical, physical, and chemical properties of bio-based products to make them viable in comparison to traditional composites, ensuring a lower environmental impact. A LCA is carried out to evaluate the sustainability of the GREEN-LOOP's development. The LCA is focused on environmental impacts from the beginning of the value chain (raw materials extraction), considering the recyclability or the waste reduction. The main project allegations say that the bio-based solutions have competitive mechanical performance with a reduction in the environmental footprint. These results talk about the potential for the materials and play an important role in the shift toward more sustainable manufacturing, providing viable solutions for industrial sectors to know environmental laws and the demand from the consumers related to the bioproducts. In summary, GREEN-LOOP Project offers innovative alternatives to traditional materials, while reinforcing the economic benefits of circular economy approaches and ensuring sustainability throughout the product lifecycle. This initiative lays the foundations for the widespread use of bio-based materials in various industries, contributing to a more sustainable and resilient future.

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5.05.P-Tu422 Life Cycle Assessment Approaches and Applications to Emerging Bio-Based Technologies

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Biomass is a promising renewable feedstock to replace conventional fossil-based products, especially in sectors that cannot be electrified such as production of chemicals, construction materials, textiles and intercontinental aviation and shipping. Some of the bio-based alternatives to replace fossil products are relatively new in the market with further improvement potential, while several highly promising options are at early stages of development (i.e., at a lab, pilot or demo scale). The application of life-cycle assessment (LCA) to emerging bio-based technologies is increasing but methodological gaps remain given their low technology readiness level (TRL). While LCA methods have been improved to estimate the environmental impacts of emerging bio-based technologies, challenges remain in ensuring the comparability and reproducibility of such assessments. This study reviews existing LCA studies, in general, and those of emerging bio-based technologies, in particular, dealing with scaling-up of technology maturity and with projection of background system to simulate industrial scale and future

socioeconomic scenarios. We identified three main challenges after reviewing 120 articles: 1) Unclear and overlapping definitions, with use of neologisms such as ex-ante or prospective, without clear differentiation of technology maturity (e.g., TRL) from temporal position (e.g., present or future); 2) varying methods for scaling-up technologies from lab to commercial/industrial scale and for modifying future background economies; 3) lack of transparency when applying Integrated Assessment Models to transform datasets (e.g., premise or any other tool). Explicitly detailing the decisions and assumptions made during both scaling-up technology maturity and future projections is key for ensuring consistency, comparability and reproducibility in LCA results. To increase clarity, we understand prospective LCA as approaches estimating future changes in background and foreground systems, mainly driven by technology and socio-economic evolutions, while ex-ante can be better understood for low TRL systems that require scaling-up of technology maturity, but background processes are not necessarily modified.

5.05.P-Tu423 Assessing Regionalization of LCI Datasets of Fossil-Based and Biodegradable Bio-Based Polymers Used for Food Packaging in the European Context

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As bio-based systems emerge as promising alternatives to FB counterparts for decarbonizing our economy, accurate LCA comparisons between fossil-based (FB) and bio-based (BB) polymers remain challenging. This study examines the influence of choosing generic versus country-specific Life Cycle Inventory (LCI) datasets on the impact assessment outcomes for key FB and BB polymers produced in Europe. Despite an increasing demand for regionalized data, site-specific datasets are often absent, and less representative generic datasets must be used. Moreover, although Europe has the greatest coverage of country-specific datasets, comparisons to generic datasets are limited in literature. The performed analysis used regionalized datasets for FB polymers (HDPE, LDPE, LLDPE, and PP) and BB polymers (PLA/starch, and TPS) from MLC Databases, applying the EF 3.1 method. Results were evaluated based on i) the occurrence of burden shifting from FB to BB polymers and ii) the influence of European country-specific datasets compared to generic (i.e., RER) datasets. Acidification and Eutrophication impact categories were found to increase in BB polymers, while a lack of standardized biogenic carbon accounting resulted to affect Climate Change assessment. Significant variations were found in Ionizing Radiation, Land Use, Ozone Depletion, and Water Use with no significant differences between FB and BB datasets. As main conclusions, the importance of regionalization in BB datasets was highlighted due to differing agricultural practices, and enhanced inventory and impact regionalization were recommended to capture regional dynamics accurately.

5.05.P-Tu424 Assessing Environmental Performance Improvements in Microalgae-based Products via the ALIGNED Framework

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The bio-based industry is important in addressing global challenges such as climate change and resource depletion. This study examines the environmental impact of a multi-product microalgae biorefinery producing omega-3-rich lipids for edible oils, protein for dietary supplements, and a protein-carbohydrate mix for animal feed. The aim of the study is to test and evaluate how the ALIGNED framework for the life cycle assessment (LCA) of bio-based products can be used in practice to assess and improve the environmental performance of microalgae cultivation and downstream processes through identifying and implementing alternatives with lower impact. The learnings shall be applicable beyond the single case to the broader microalgae aquaculture sector. Several methods and tools developed in the ALIGNED project framework were implemented, in particular the methods for prospective background modelling (LCI), the methods for biogenic carbon accounting (LCIA) and for uncertainty analysis (interpretation), and methods for data structure and input (LCI), among others. The study evaluates a base scenario alongside alternative scenarios, such as the integration of on-site photovoltaic energy supply. Prospective analyses are conducted to explore future scenarios using predictive models. This is especially relevant as the case study represents an emerging industry. Results of the specific case considered show that the cultivation process has the largest environmental impact compared to harvesting and biorefinery, electricity being the main contributor to this impact, along with carbon dioxide and nitrogen source supply. The ALIGNED framework has provided tools accessible to non-experts, although further work can still be done to enhance the accessibility and simplify the experience.

5.05.P-Tu425 Life Cycle Costing Tool for Assessing Economic Impacts of Bio-based Products and Systems

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Innovative Bio-based Systems (BbS) are set to play a role in reducing dependence on non-renewable resources, manage natural resources sustainably, mitigate and adapt to climate change, strengthen European competitiveness, scale up biobased sectors and unblock investments. However, when evaluating the substitution effects of this transition, current Life Cycle Sustainability Assessments (LCSA) often miss significant economic impacts when referring to BbS. The methodologies that are currently being developed mainly focus their economic assessment in industrial processes and their manufacturing stage, overlooking the costs of other relevant stages of a sustainability assessment such as the use phase or the end of life (EoL). Incorporating all the life cycle phases provides a better understanding of the economic benefits of transitioning from fossil-based to bio-based systems, facilitating easier comparisons between the two and allowing the inclusion of the economic assessment in the LCSA, marking a methodological advancement for conducting Life Cycle Costing (LCC) to BbS.

In this context, there is a need to integrate comprehensive economic indicators into LCSA frameworks to evaluate the total economic impact of a bio-based product or system over its entire life cycle. In addition, trade-offs in dynamic carbon storage considering GHG emission dynamics over time must be assessed and included in the economic evaluations. To address this issue, a selection and a harmonization of methods scientifically sound to assess LCC on BbS is performed to enable accurate evaluation of the potential economic impacts of bio-based products and technologies. LCC also enables the comparison of different production methods, feedstocks or EoL scenarios, optimising the economic impacts.

The development of the LCC user-friendly tool is underway through stakeholder engagement. Key stakeholders were identified and invited to participate in co-creation processes, ensuring that the resulting tools are accessible, and the indicators are appropriate and practical for end-users. This tool will form part of a broader suite designed to perform Life Cycle Sustainability Assessments (LCSA) for BbS.

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5.05.P-Tu426 Towards the Integration of Bioeconomy Constraints in Life Cycle Assessment

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The transition to an EU-level bioeconomy will induce a growing demand for biomass feedstocks. The extent to which the production of different biomass feedstock types can respond to the increased demand is limited by many factors. This leads to competition between sectors demanding the same, finite biomass feedstocks. Considering these constraints and competition when conducting a Life Cycle Assessment (LCA) of bio-based technologies is fundamental. We developed a framework that allows to construct marginal market mixes for bio-based products and feedstocks by adapting EU-wide data on biomass production and use for applications in LCA.

We use an EU JRC dataset on biomass production, supply, uses, and flows. The dataset allows for a comprehensive cross-sectoral representation of the EU bioeconomy. It provides detailed biomass flows linked via supply and use data across different sectors. The cross-sectoral mass-based data is used to investigate seven bioeconomy sectors and capture the indirect effects of the corresponding fossil sectors on their markets.

Through consequential LCA, using JRC-derived economic data and policy trends, we forecast the future development of the supply and identify the unconstrained production activities, those with the capacity to increase production that will respond to the increased demand. We then create new market activities that represent the projected share of these activities in the marginal mix for biomass feedstocks.

We represent the seven targeted sectors and the relevant marginal mixes in a matrix format which enables the integration of the proposed model with LCI databases, allowing to capture the competition for biomass between different bio- and non-bio sectors in LCA.

The framework allows to obtain more holistic assessment of the environmental impacts linked with the shift to bioeconomy by computing new marginal mixes that capture the indirect burdens of competing bio-based and fossil sectors. Further work will focus on expanding the framework beyond bio-based products.

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5.05.P-Tu427 Density and Moisture Content Impact on the Environmental and Economic Performance of Woody Feedstock

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Background: Climate change is accelerating and our society is running out of time to stop us from exceeding our planetary boundaries and irreversible and dramatic consequences for life on our planet. Biomass is considered to be one of the solutions. Wood as a sustainable and regenerative source can be utilized for different applications within different bio-based sectors and technologies.

Challenge/problem statement: While the focus is usually on developing and optimizing novel emerging technologies, the woody feedstock and its environmental and economic implications are calculated very basic, potentially missing further optimization possibilities. The unique biological and physical characteristics of wood are often neglected. Particularly, the density and the moisture content of wood are very much species-specific affecting several (life cycle inventory) activities (e.g. drying, transportation, and process system engineering).

Method: Hence, the life cycle and techno-economic assessments are further extended by the dynamic density and moisture content model to account for feedstock-specific characteristics to further explore optimization potentials.

Results: The assessment shows that carbon emissions and costs decrease with increasing moisture content in feedstocks. Furthermore, the implications of higher moisture content on the performance of biorefineries were discussed, indicating that it has the potential to decrease carbon emissions and increase the economic feasibility of the biorefineries.

Summary and relevance: The newly developed extension enables to further optimize the utilization of feedstock by decreasing the cost and carbon emissions of feedstocks and biorefineries, giving the technology developers the option to develop technologies more flexibly.

5.05.P-Tu428 Towards Alternative Fertilising Products PEFCR: From Methodological Hotspots Identification to Industrial Case Studies Validation

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Alternative fertilisers derived from secondary nutrient raw materials are pivotal for the Circular Economy while aligning with EU policies like Farm-to-Fork and new fertilising regulations. These products, sourced from agricultural, livestock, urban, and industrial waste, enhance Europe's economic resilience, promote resource efficiency, and support decarbonisation efforts. However, their environmental performance relative to mineral fertilisers remains uncertain due to the absence of standardised assessment frameworks.

The EU's Product Environmental Footprint (PEF) offers a harmonised Life Cycle Assessment (LCA)-based approach, yet no specific PEF Category Rules (PEFCR) exist for fertilisers, particularly bio-based alternatives. Addressing this gap, this work proposes a PEFCR-wise for alternative fertilisers. The process included an extensive literature review of LCAs, complemented by bilateral meetings with relevant stakeholders (fertiliser industry and JRC) and online surveys with industry leaders and practitioners. The proposed PEFCR-wise method was tested with data from 25 fertilising products derived from diverse raw materials such as blood, animal excreta, and agro-industrial waste. Preliminary results highlight key methodological challenges, including the selection of an appropriate functional unit (FU) and allocation rules. Stakeholders generally agree that relying solely on mass-based FUs is insufficient for fertiliser benchmarking, though no consensus on a universal FU has been reached.

To conclude, the first PEFCR for alternative fertilising products has been developed and applied to achieve the most suitable LCA methods for alternative fertilising products. Moreover, these methods were tested by a relevant share of the European fertilizer industry. Nonetheless, there are challenges mostly related to credits and burdens allocation along the supply chain, the precise responsibility splitting and the consequences of defining the feedstock as a waste or a product, and the emissions derived from the application technique or the multifunctionality in the supply of different nutrients by compound fertilisers.

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5.05.P-Tu429 Assessing Environmental Impacts in Single Cell Protein Production: A Prospective LCA Approach

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Transforming food production systems is essential to ensure that the use of natural resources and emissions remain within planetary boundaries. Innovative protein sources, such as Single Cell Protein (SCP) derived from the biomass of unicellular organisms, are emerging as promising solutions to address food scarcity and meet global nutritional needs. This study presents a prospective life cycle assessment (LCA) of different scenarios for SCP production using biogas derived from fish industry waste, with a focus on upscaling a lab-scale process. Four configurations were compared, exploring the recovery of heat, electricity, fertilizer, protein, and biomethane. The recovered materials and energy were included by expanding the system boundaries to account for avoided primary production, thus mitigating the environmental burdens associated with their production.

The results reveal significant differences in environmental impacts between laboratory-scale and industrial-scale processes. When upscaled, the environmental impacts were lower compared to other protein sources, highlighting the environmental benefits of protein derived from biomass cultivated with biomethane obtained through anaerobic digestion of fish industry by-products and waste. The baseline scenario showed the poorest environmental performance due to biogenic methane emissions from unutilized biogas. In contrast, modified scenarios incorporating various biomethane utilization pathways demonstrated substantial reductions in all impact categories. Among these, Scenario 3, which recirculated energy from cogeneration for internal use, showed the best environmental performance. Overall, the findings suggest that the optimal configuration combines biomethane, heat, and electricity recovery, providing valuable insights for further industrial-scale applications. Future challenges will focus on technical and economic feasibility, particularly in terms of technology readiness level, implementation, and investment.

5.06.P Including the Biogenic Carbon Emissions and Removals in Life Cycle Assessment: Advances and Challenges

5.06.P-We415 Biogenic Carbon Accounting and in Life Cycle Assessment of Single-Use Plastic Alternatives

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The shift from fossil-based plastics to bio-based alternatives raises important questions regarding the scale of alternative material demand and associated environmental impacts. This study aims to comprehensively evaluate the implications of increased demand for bio-based materials, initially focusing on paper and paper-based products in North America, arising from policies banning the use of single-use plastics. Through a critical review of single-use plastic policies and data on alternative material substitutions, we analyse market-driven demand for bio-based materials, estimating feedstock and land use requirements resulting from expanded production. We also assess existing policies on plastic ban and biogenic carbon accounting methodologies to identify potential advantages, trade-offs and unintended consequences. The study explores the current capabilities and limitations of life cycle assessment methodologies in capturing biogenic carbon impacts considered for the first time in relation to bio-based alternatives for single use plastics- through a case study of corrugated cardboard packaging product. Carbon flows are estimated based on a critical review of published studies and statistics pertaining to paper and cardboard production; product lifetime; end of life management; and retention of biogenic carbon within the product system via recycling. We apply a range of biogenic carbon assessment methodologies (0/0; -1/+1; dynamic; and GWPbio) to assess climate change impacts. Preliminary results provide insights into land requirements, greenhouse gas emissions, and key environmental indicators where notable differences may arise between fossil-based plastics and bio-based alternatives. The findings aim to support more informed policy development and decision-making regarding single-use plastic bans, laying the groundwork for broader assessments of other materials and regions in future research phases.

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5.06.P-We416 From Macronutrient Content to Biogenic Carbon emission: Advancing Biogenic Carbon balance in Life Cycle Databases

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Biogenic carbon emissions and removals, encompassing carbon fluxes from biomass, land use and land-use change, and carbon storage in products, play a critical role in the global greenhouse gas (GHG) balance. These processes are vital for achieving climate mitigation targets, conserving biodiversity, and supporting renewable resource provision. However, integrating these dynamics into life cycle assessment (LCA) remains challenging due to gaps in standards, methods, and data, resulting in inconsistencies in modeling biogenic carbon flows. In this context, getting biogenic carbon properly balanced in the dataset (input-output) became a crucial point to improve biogenic carbon accounting in LCA.

Ecoinvent is a worldwide established LCA database that accounts for around 20.000 datasets modelling human activities and processes and includes key sectors for biogenic products such as agriculture, forest, pulp, and paper. To quantify biogenic carbon in biomass growth, properties of elementary exchanges, Carbon dioxide, in air (resource from the air) which is the uptake by biomass growth, and Carbon dioxide, non-fossil as biogenic releases, are relevant. To improve biogenic carbon accounting in dataset harmonized modelling is needed in ecoinvent. The uptake should correspond to the carbon in products and byproducts leaving the process (for example, crop and straw) minus the carbon in relevant products entering the process (for example, seed and seedlings). Biogenic carbon of intermediate and elementary exchanges is quantified by the mathematical relation between dry mass and carbon content property. Dry mass quantification can be calculated using water or moisture content, while biogenic carbon by its average or specific percentage in lipids/proteins/carbohydrates/ash and fibre added as new properties in each specific exchange. Carbon in kg fixed in biomass can be estimated by multiplying the dry mass (in kg) by the carbon content (kg/kg).

Harmonized inventory for the bio-based (mainly agricultural) sectors in ecoinvent shows increased biogenic carbon accounting at the dataset and database level regarding carbon storage in biomass growth and significant variation in Carbon dioxide, in air and Carbon dioxide, non-fossil amounts for balancing.

5.06.P-We417 Investigating Approaches to Model LUC Impacts of EU Consumption

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The EU is a large importer of virtual land embodied in biobased commodities. As a consequence, EU consumption drives land use change (LUC) taking place overseas and is responsible for well-known related environmental pressures and impacts including deforestation, biodiversity loss, and contribution to climate change. The goal of this study is to investigate the influence of different calculation approaches and methodological choices on the assessment of the impact on Climate Change caused by LUC deriving from European consumption. To this end, it builds on a recently developed model that estimates the cropland, grassland and forest land embodied in EU consumption of biobased products, through a physical based accounting approach built on statistical data on production and trade of over 500 commodities. These virtual areas are then converted into emissions from LUC by means of statistical LUC (sLUC) approaches. Two different calculation approaches are used: the one outlined in the international standard PAS2050 and in the draft version of the GHG Protocol Land Sector and Removals Guidance.

A number of alternative methodological approaches are tested: when applying the PAS2050 two different approaches are used to calculate the average land use change associated to a crop (i.e. normal and weighted average taking into account the country's changes in land use categories), while in the approach proposed by the GHG Protocol two alternatives are used to allocate the national land use change to a crop (product expansion and shared responsibility) and two different time discounting approaches are applied (equal and linear). The resulting emissions are compared, and differences are discussed.

The present analysis is being conducted in the context of the update of the Environmental Footprint methods, the LCA impact assessment method recommended by the European Commission, for what concerns the modelling of emissions from land use change. Our analysis helps to shed light on the implications of these choices when using modelling results in support to policies. Due to its large scale and significance, our work is also relevant to support the design, development and implementation of future policies.

5.06.P-We418 A Time-Explicit Life Cycle Assessment of Woody Biomass Utilisation Scenarios in Switzerland

Arthur Jakobs, Paul Scherrer Institute (PSI), Switzerland

Holistically assessing the climate impacts of forest product utilisation is a complex endeavour, as it requires the integration of diverse and interdependent factors across spatial and temporal scales, from forest growth and disturbances influenced by changing climate scenarios to forest management practices that further determine the availability and characteristics of harvested wood as well as the change in the

forest carbon stock. Additionally, the end-use scenarios of wood products, encompassing potential carbon storage and substitution effects, must be evaluated in the context of a decarbonising economy. Addressing the multitude of these interconnections demands a comprehensive framework capable of capturing the full scope of the life cycle of a wood product, from carbon sequestration in the forests to the final release of the carbon, potentially decades.

We present a framework that combines forest management scenarios and dynamic material flow analysis to inform an Energy System model for Switzerland. This energy system model, in turn, is integrated with socioeconomic scenarios from Integrated Assessment Models to create prospective databases using Premise, that are subsequently used to perform time-explicit (dynamic prospective) life cycle assessments (LCAs) using *bw_timex* of various wood utilisation scenarios. This approach enables a detailed comparison of the opportunity costs of these scenarios, accounting for temporal storage, potential cascading uses of wood products, evolving background economies, and forest growth dynamics. This study highlights the importance and challenges of coupling forest dynamics with downstream utilisation scenarios and broader economic contexts to evaluate the trade-offs and synergies in forest product utilisation. By addressing the temporal and systemic complexities, our framework advances the holistic assessment of forest-based bioeconomy strategies, offering a robust tool for assessing woody biomass utilisation.

5.06.P-We419 Comparison of Biomass-Based Synthetic Natural Gas Production Scenarios: Cradle to Gate Life Cycle Assessment

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In studies comparing the environmental impacts of renewable fuel production using biomass feedstock with fossil-based alternatives, the analysis often focuses on net greenhouse gas (GHG) emissions, excluding biogenic carbon dioxide emissions. However, this neglects the high carbon intensity of biomass-based processes, a factor often overlooked when calculating net GWP emissions, as suggested by the IPPCC.

To fill this gap, this study considers CO₂ biogenic emissions and relevant impact categories affected by the use of biomass such as acidification potential, eutrophication potential, particulate matter, land use and water use, following the environmental footprint 3.1 method.

The aim of this study is to provide a comprehensive overview, identify the key environmental hotspots of different scenarios, and propose areas for improvement.

For this a cradle to gate life cycle assessment is (will be) performed using experimental and simulation data from previous studies.

The study highlights the importance of renewable electricity sources and CO₂ capture in tackling GHG emissions. It also shows that the choice of feedstock can significantly impact other environmental categories, such as land use, eutrophication, and acidification, emphasizing the need for sustainable farming practices and the development of technologies aiming to use residues and waste as feedstock. Most importantly, by calculating the biogenic CO₂ emissions the study shows that these are not negligible. Therefore depending on how biogenic CO₂ emissions are accounted for, these technologies could emit more CO₂ than current systems, reinforcing the need to focus on reducing the carbon dioxide stock in the atmosphere.

The results of this analysis will contribute to decision making by highlighting the most promising directions for the development and improvement of these technologies.

5.06.P-We420 Exploring Multiple Approaches to Biogenic Carbon Assessment: A Comparative Life Cycle Assessment of Wooden and Resin Flooring in the Nautical Sector

Federico Bedogni, Eleonora Rossi, Francesco Arfelli, Daniele Cespi and Fabrizio Passarini, University of Bologna, Italy

Despite significant strides toward sustainability, in 2018, the maritime sector was responsible for approximately 3% of global greenhouse gas (GHG) emissions and around 13% and 15% of SO₂ and NO_x global emissions, respectively, mainly due to fuel consumption. Still, the focus on the environmental impacts of ship materials has been increasing recently. This study aims to conduct a comparative life cycle assessment (LCA) of two boat flooring alternatives, i.e., one wooden-based (teak) and one fossil-based (resin), considering their entire life cycles, thanks to primary data provided by the manufacturer for the wooden-based material. The main focus, beyond comparing the two alternatives, was to assess biogenic carbon and its uptake using various approaches. This is particularly relevant because different methodologies can lead to significantly varying results, demonstrating several shortcomings and blindness, heavily influenced by the end-of-life scenario (landfilling or incineration). Two standard procedures for long-term emissions (ISO 14064 and IPCC) were considered, alongside the 0/0 and -1/+1

approaches, shaping different use phase scenarios for treated and non-treated wood panelling. The selected functional unit (FU) is 1 m² of flooring material, and a cradle-to-grave approach was adopted. The LCA modelling was done using SimaPro software (v.9.5), with the ecoinvent database (v.3.10) applying IPCC 2021-GWP100 as the analysis method for Climate Change (CC) contribution and ReCiPe 2016 for all the other midpoint categories. The results show that wooden products have a lower impact than resin in every scenario, even considering long-term emissions, regarding CC, particulate matter formation, human carcinogenic and non-carcinogenic toxicity (not land use) which represent 98% of the total contribution on the ReCiPe 2016 single score (perspective H). The geographical and temporal distribution of these regional impact categories contribution is also commented on. A mitigation of the overall impacts thanks to energy and heat recovery during waste to energy, especially concerning the woody fraction, is observed. A general increase in the impacts can be observed in the treated wood panelling with respect to the non-treated one. The emission profile over time was evaluated to improve the monitoring of CC contributions and to better understand possible actions for their mitigation, process not obtainable through traditional calculation tools.

5.07 Life Cycle Assessment Relevant Resource Indicators for Providing Guidance Towards a Transition to a Resilient Carbon Neutral and Circular Economy

5.07.T-01 Integrating physical material quality of recycled materials as part of the life cycle inventory in life cycle assessment

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The EU is implementing circular economic policies to reduce the environmental burdens of its material economy. A key pillar of circular economy is recycling which is proposed as one of several material strategies in the Packaging and Packaging Waste Regulation. A research gap within recycling in LCA is understanding how to address physical material quality when developing life cycle inventories of recyclable and/or recycled materials. Recent research demonstrates that additives, contaminants, inks, and other substances have a negative influence on the material quality, which again affects the market applicability of recycled material and how many times a material can be recycled. Both of these aspects affect the usable recycled output at a product level, and the net recycled content across a recycling cascade and subsequently at the market level.

This research aims to integrate emerging research on the physical effects of additives, contaminants, inks, and other relevant substances on recycling into a life cycle inventory modelling approach for addressing its environmental influence on recyclable or recycled materials. To do so, a multi-scope and multi-method approach is applied to integrate the consequences of material quality on the material flow between product systems within a recycling cascade and its extended effects on and from the market material economy. Current literature states that it is not necessarily environmentally beneficial to only increase the recycling rate of recyclables. Rather, there is a need for a market that fosters the inclusion of recycled and recyclable materials. This research will find how material quality affects the recyclability of plastic materials as well as identify which, and how much, recyclable plastic can be used. Further, the results will demonstrate the physical and environmental effects of the material flow of recyclables through a material economy. These results provide a method for integrating material quality of recyclables as part of the LCI when addressing recycling and recycling systems.

5.07.T-02 A Change of Paradigm on Mineral Resources Flows in LCA: Necessary, and Feasible

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The assessment of impacts associated with mineral resource use has long been debated among LCA method developers and practitioners. Several methods (including the EDP, ADR/LPST, ARP, JRC-LCI, CTI-LCIA and EVDP methods) have been developed in the recent years to go beyond the assessment of contributions to resources depletion over a product life cycle in LCA, and to rather assess contributions to reduction of resource accessibility and resource dissipation. This study builds on, and extends, a review on the fundamentals of these recently developed methods, including key underlying choices and assumptions, and operationalization. It moreover analyses the terminology associated with material flows in LCI modelling, and discusses the implementation of material balancing in process engineering and Life Cycle Thinking.

This study highlights the two pivotal aspects that make a change of paradigm on mineral resources in LCA necessary. Firstly, some recently developed methods pledge for new LCIs to be developed (JRC-LCI method), or require the development of new LCIs (EVDP, CTI-LCIA) for any potential wide implementation. These new methods call for a deep change of paradigm on modelling mineral resources in LCIs, no longer considering only primary mineral resources extracted from ground, but also mineral

resources flows within the technosphere, or from the technosphere to the ecosphere. Secondly, rethinking mineral resources flows in LCIs is necessary to support the development of material-balanced LCIs. The latter contribute to more consistent and complete accounting of intermediate exchanges, emissions and resources flows at the unit process level.

Moreover this study develops a stepwise approach to make this change of paradigm feasible in LCA practice. This approach integrates material accounting and balancing principles in LCI results, enabling to derive balanced LCI datasets and databases. It builds on a new nomenclature of mineral resource flows, not only extracted from ground but also exchanged within technosphere, and from technosphere to ecosphere. This framework is showcased with a step by step implementation to the case of the production of a battery raw material, using ecoinvent datasets. The influence on toxicity- and resources-related impact categories is discussed, particularly highlighting the benefits gained from this framework towards sounder support to decision-making with LCA.

5.07.T-03 Scenario-Specific Characterization Factors of Dissipative Flows of 53 Abiotic Resources: Introduction to the ACP and RESEDA Methods

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Abiotic resources such as fossils, metals or non-metallic minerals are essential to industrialized societies. Through their use in multiple products, they provide services such as mobility, conditioned living space or worldwide instantaneous communication. The extraction and the transformation of these abiotic resources made available in the technosphere into different products enable human society to access to these services. Losses of these resources along their life cycle (e.g. primary production, use phase or recycling), here called dissipative flows, can potentially reduce the provision of those services. To meet the global demand of services, additional extraction flows may be required to compensate the dissipative flows. This additional extraction requires an supplementary energy consumption. We developed the Additional energy Cost Potential (ACP), a life cycle impact assessment method which quantifies an additional energy cost of a marginal dissipative flow (in MJ per kg of dissipative flow), integrated over time (from now to 2100) and space (global level). Characterization factors of dissipative flows range from 1.75E5 MJ/kg dissipative flow of Platinum to 1.3E-1 MJ/kg dissipative flow of iron. Moreover, there is a maximum yearly amount of metals that can be extracted given the capacity of worldwide mines to extract. If the global demand is superior to the global mining capacity, users of a resource may face a deficit as the products providing a service cannot be produced. We introduce a complementary life cycle impact assessment method, named REsource Services Deficit Assessment (RESEDA) method which quantifies the deficit quantity (in unit of mass) resulting from a marginal dissipative flow. This corresponds to the situation where the dissipative flow cannot be compensated by an extraction flow, as demand is above mining capacity. We determined characterization factors of dissipative flows of 50 metals and three fossil fuels (crude oil, natural gas and coal) for each scenario with the RESEDA method. Characterization factors of dissipative flows range from 0 kg deficit/kg dissipative flow of iron to 0.93 kg deficit/kg dissipative flow of cobalt.

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5.07.T-04 Spatially and Temporally Differentiated Characterization Factors for Supply Risk of Abiotic Resources in Life Cycle Assessment

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Life cycle assessment (LCA) is a commonly used method for evaluating environmental impacts throughout a product's life cycle, generally focusing on "inside-out" impacts such as resource consumption and global warming. However, the "outside-in" perspective, which considers the availability and accessibility of resources to industry, is gaining more attention. This approach incorporates raw material criticality assessments into LCA, offering a broader view of supply risks. The GeoPolRisk method evaluates geopolitical supply risks by examining factors like production concentration, import shares, political stability, and commodity prices. This article introduces a characterization model for GeoPolRisk, calculating the Geopolitical Supply Risk Potential (GSP) for 46 raw materials across various countries and years. The results reveal significant variations in GSP values, particularly for precious metals like platinum group metals, which are produced in politically unstable regions. The case study on energy

supply security illustrates how the GeoPolRisk method provides valuable insights into the supply risks of critical raw materials, complementing traditional environmental indicators like global warming in LCA.

5.07.T-05 Holistic Sustainability Assessment of Packaging – the Viennese Model of Sustainability Assessment

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The regulatory landscape for packaging in Europe is changing rapidly. The recently adopted Packaging and Packaging Waste Regulation (PPWR) and the Corporate Sustainability and Reporting Directive (CSRD) set new requirements on packaging sustainability. Standardisation and industry wide cooperation along the supply chain is necessary to fulfill these requirements.

For sustainability assessment of packaging a holistic model is required to include all relevant sustainability indicators and prevent burden shifting. The Viennese Model of Sustainability Assessment was developed together with companies along the supply chain to select KPIs and methods for a holistic sustainability assessment including circularity and product protection that fulfills the upcoming regulatory requirements. A benchmarking study of dairy packaging currently on the European market shows that a significant number of packaging formats are not fit for 2030 being either not recyclable or showing high environmental burdens. Holistic sustainability assessment combining circularity, environmental and product protection Key Performance Indicators is essential to identify opportunities for optimisation, prevent burden shifting and monitoring environmental performance of packaging. Limited emptiability of packaging, an often overlooked indicator can have very large impact on environmental performance and contributes significantly to food waste and should be prevented by design changes.

5.07.P Life Cycle Assessment Relevant Resource Indicators for Providing Guidance Towards a Transition to a Resilient Carbon Neutral and Circular Economy

5.07.P-Tu430 Understanding the Production of Natural and Synthetic Battery-grade Graphite: European Union Perspective on Supply Risk and Environmental Impact Implications

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Lithium-ion batteries are vital for e-mobility, with graphite as a key anode material. As e-mobility grows, demand for battery-grade natural graphite (NG), classified as a critical raw material for the European Union (EU) and synthetic graphite (SG) will rise. Though NG and SG have similar structures, they differ in origin, production processes, properties, and cost. Both involve energy-intensive production with potential environmental impacts, raising concerns in the EU, which aims to cut emissions and strengthen supply chain resilience. This study evaluates environmental impacts, with a focus on climate change (CC), and supply risks for battery-grade NG and SG to align with the EU's goals for resilient and sustainable battery production.

Environmental impacts are assessed using Life Cycle Assessment with 1 kg of battery-grade graphite as the functional unit. Reliable LCI datasets are remodelled using ecoinvent 3.10 for background data. Two production routes, NG and SG, are analyzed. Supply risk is assessed using the GeoPolRisk method from an EU perspective, focusing on flake graphite and needle coke, as well as covering raw materials in the background system.

Preliminary results show that producing 1 kg of NG results in a potential CC impact of 5.4 kg CO₂eq., while SG production results in 10.3 kg CO₂eq. GeoPolRisk scores for the EU are 0.3 for flake graphite and 0.05 for crude oil (a preliminary proxy for needle coke). While NG's lower CC results supports EU sustainability targets, its reliance on Chinese production raises supply risks unless new deposits are developed or stable trade agreements are secured. SG presents lower supply risk but higher CC results due to energy-intensive processing.

Neither NG nor SG is expected to dominate the anode market, both will play roles in sustainable battery development. This study integrates supply risk assessment into LCA and provides GeoPolRisk scores for compounds beyond raw materials, offering insights into trade-offs between environmental impacts and supply risks to support the EU's sustainable mobility goals. It also provides a foundation for scenario development, considering variables like recycling rates or new technologies.

5.07.P-Tu431 Integrative Review of Circular Strategies: Developing a Value Assessment Framework **Jannie Coenen, Lowik Pieters and Johannes Lijzen, RIVM Dutch National Institute for Public Health and the Environment, Netherlands**

Over the past decade, nations including Brazil, China, Canada, EU countries and the US have worked on policies for a circular economy (CE). These mention high-quality recycling as essential strategy for a CE, but do not specify which (quantitative) metrics define quality of secondary materials. The lack of a definition and indicators limits comparing strategies, and measuring and evaluating the progress of the transition towards a CE.

With an integrative literature review in progress, we aim to come to a solid framework that incorporates a range of metrics for assessing quality in terms of values associated with processing and the application of secondary materials across multiple use cycles. The scope of the study is abiotic and biotic waste management. We focus on the post-use R-strategies reuse, repair, repurpose, refurbish, remanufacture, recycle, and regenerate, all aimed at bringing materials into a new usage cycle. Consequently, energy recovery and strategies to reduce primary inputs are not included in this analysis.

A search was carried out in Scopus and Web of Science with search string: (TITLE-ABS-KEY (circular* OR closed-loop* OR revers* supply chain*) AND TITLE-ABS-KEY (value creat* OR value retent* OR value preserv* OR value maint* OR quality of recycl*)). This search yielded a total of 5,380 results. We used three a priori criteria for inclusion: 1) relation to the circularity, closed-loop or reverse supply chain research field; 2) inclusion of post-use R-strategies; 3) inclusion of quantification of value and if yes: minimum two different value aspects (environmental, social, economic, technical). The large number of records was initially analysed using machine learning-assisted software, which evaluated titles and abstracts against the established selection criteria. Subsequently, 416 approved records underwent manual verification by a different researcher. To date, we have selected and analysed metrics from 25 papers, and we are currently in the process of reporting and comparing equations and parameters.

Initial findings yield 34 metrics. The majority pertain to environmental quality, while several quantify technical quality and efficiency. Some metrics encompass other socio-economic metrics, such as job creation and health impacts. Commonly used methods are life cycle assessment, multi-criteria decision analysis and mixed-integer linear programming. We observe that metrics often overlook the dynamic interactions among parameters.

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5.07.P-Tu432 An Integrated Approach to Quantify the Life Cycle Impacts and Circularity of the Digital Infrastructure

Thomas Hennequin, Aaradhya Bansal, Martijn Kamps, Ivan Vera Concha and Felipe Blanco Rocha, TNO, Netherlands

Digital services, such as video streaming, artificial intelligence, or office software use, tend to appear as immaterial, the so-called cloud. Actions are performed with the touch of a screen, or the click of a mouse, while the supply chains that enable these services are invisible to end users. The consumption of digital services is increasing globally. Corporate and governmental strategies increasingly include digitalization as a sustainability measure. Yet, digital services are not immaterial or invisible, they instead rely on material- and energy-intensive infrastructure to transmit, process, and store data. We lack a clear understanding of the environmental consequences of this growing digital infrastructure.

Scientific research on the environmental sustainability of digital services and infrastructure faces several main challenges: (1) data that is scarce and lacks transparency, (2) carbon tunnel vision with a focus on use phase energy consumption, omitting other impacts, and (3) truncated system boundaries, often overlooking end-of-life of e-waste. Only by addressing these challenges can we provide transparent advice needed to guide user behavior, corporate decision-making, and policy design.

In our work, we aim to develop an integrated assessment model in an effort to overcome these challenges. We will evaluate the life cycle greenhouse emissions and circularity of key groups of information and communication technology products and their connection to the rest of the global economy. Our approach allows for the modelling of interventions, such as EU policy measures, novel circular technologies, or sustainable data centers, and provides insights into the mitigation potential of such interventions. Our hybrid methodology combines bottom-up and top-down approaches and is rooted in prospective life cycle assessment and dynamic material flow analysis.

With our results, we will provide a robust and transparent overview of the digital infrastructure and its impact on climate change and material scarcity. We aim to show the evolution of impacts over time and gain insights into the link between the digital infrastructure and the rest of the economy. We will also reflect on the usefulness and limitations of the different methodological tools that were utilized. Finally, we aim to provide recommendations on the most effective strategies to sustainably use digital services.

5.07.P-Tu433 How to Assess Impacts on Biodiversity of Metal Sources? Providing Indicators for Decision-Making

Anne Asselin¹, Joséphine Gatin¹, Chunqi Hui³ and Ambre Brizon³, (1)Sayari, France, (3)L'Oreal, France
Metal mining and refining are sources of impact on biodiversity, through land (and potentially sea) use change, pollution, and climate change. Major impacts occur at the two first steps of the value chain, namely ore extraction and smelting. Businesses are looking for simple yet robust criteria to define and improve their sourcing. Metals production can indeed impact biodiversity within diverse ecosystems within the three biotas: terrestrial, freshwater, and marine.

Building on existing literature, and based on a life cycle approach, we developed a semi-quantitative assessment method for metal sources, prioritizing the most important cause effect chains. As for the method, we i) first identified three metals of interest with diverse technical extraction practices ii) did a literature review, gathering information on main mining spots and practices, as well as on pressures on biodiversity caused by mining iii) identified the main drivers (land and water use, metal pollution) and built a semi-quantitative method based on cause-effect chain, with resulting rating on a scale from 1 (very good) to 7 (very bad) iv) completed our work with a quality assessment of the rating v) tested it on 15+ case studies.

Our method assesses impacts on biodiversity from water and land use, heavy metal pollution, and GHG emissions. It covers terrestrial, freshwater, and marine biota, and combines the magnitude of each pressure with the receiving ecosystem vulnerability, using the Global Extinction Probability combined with assessment of governments integrity and environmental policies. We have been testing it on 15 mining and refining facilities and are currently further expanding the testing especially for copper and cobalt in China and DRC. Those case studies present various challenges e.g., allocation between coproducts (in the case of copper and cobalt in DRC); scarce and incomplete data on metal pollution; local and generic data; aggregation of marine, freshwater and terrestrial impacts, consistency of available data from various sources (governments, companies reporting, NGOs)

Project is still ongoing; results of testing will be presented at the conference. We will also shed light on practical data collection and quality and discuss how to provide actionable yet robust indicators for business decision-making.

5.07.P-Tu434 TOC/TN Measurement for the Control and Evaluation of Methane Fermentation of Food Waste

Yoshio Ikezawa, Shimadzu Corporation, Japan

Methane fermentation is a biological process in which microorganisms decompose organic materials such as food waste in the absence of oxygen. Methane gas generated through the process is a renewable energy source and fermentation residue can be used as fertilizer. In Japan, a research laboratory have been developing a recycling system which food processing companies adopt methane fermentation plants. In the investigation to make this process improvement, Total Organic Carbon analyzer with Total Nitrogen detector has been applied because the ratio of TC and TN is pretty relating to the efficiency of the process of methane gas generation. This presentation will show how TOC and TN measurement is working for the optimization research process development.

5.07.P-Tu435 Facilitate Decision Making in LCA Introducing the Measure of Resource Efficiency

Antoine Beylot¹, Guido Sonnemann² and Louis Freboeuf², (1)Bureau de recherches géologiques et minières (BRGM), France, (2)Université de Bordeaux, France

The presentation will first describe the meaning of measuring Resource Efficiency in Life Cycle Assessment (LCA).

Reminding the different resources that are, or that could be inventoried in LCA, and the different way of characterizing them in regard to the safeguard subject identified by the GLAM 2024. The consequences are conceptually described as The consequences can be the requirements of additional efforts to compensate lost values or the lost values per se as externalities of natural resource use.

Then the notion of circularity is introduced with its importance for human growth [1], its interest for economies, for instance in the Circular Economy Action Plan (CEAP) of the European Commission among other regional initiatives [2,3]. The interest of accounting for the reversibility of a system flows are presented as an interest for LCA to resolve multifunctionality in process-oriented studies [4], and physical balance [5] issues in traditional attributional LCA studies. Circularity initiatives for circularity metrics implementation are presented [6].

Then, a metal recycling system, at the heart of the Circular Economy (CE) state, will be assess using different implemented resources CE metrics. The different metrics will be grouped in function of their decision-making insights. The causes of their differences will be assessed. Based on these insights, a proposing of considerations for the incorporation of Resource Efficiency measuring in the LCA

framework will be presented.

The case study's data exploited come from [7].

This presentation subscribes in the dynamic of normalizing circularity assessment techniques in the framework of life cycle thinking. Indeed Life Cycle Assessment (LCA) is considered as a promising tool according to the last version of the (International Organisation for Standardization, 2024) [8].

5.07.P-Tu436 Developing a Comprehensive Framework for Optimal Circular Cascading Use of Wood: Sustainable Criteria and Application Selection

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Wood, a versatile and renewable material, plays a significant role in various industries and is a key resource for the bioeconomy. Forests and sustainable wood use are expected to be essential in reaching net-zero goals by sequestering CO₂ in biomass, storing CO₂ in materials, and substituting fossil resources. However, a systemic understanding of wood's life cycle complexity is required to defossilize society and promote circular bio-based developments. By recognizing wood as a complex material, with changing properties throughout its lifespan, we developed a material flow analysis (MFA) method covering the entire Swiss wood value chain -from harvest to products, societal use, collection, recycling, energy generation, and trade. We now expand on this work by integrating life cycle assessments (LCA) and identifying pathways to maximize the duration and environmental efficiency of biomass use in the technosphere and investigating the cascading use of wood, where wood is (re)used as long as possible before energy recovery. We identified underutilized wood streams - such as damaged wood, residual wood, wood fuel, and waste wood with initial potential for cascading use, as they meet the required criteria of availability, being untreated, and having potential for reuse or recycling. We will now develop "material-first" scenarios to maximize the use of these flows as materials. This includes considering future changes in wood availability from forests and the introduction of new emerging applications and reused or recycled materials. To establish material cascades we further characterize the initially identified flows (e.g. by size/form, carbon content, contamination) and elaborate corresponding quality criteria for the wood flows to determine pathways of wood material (re)use. Finally, we will identify suitable applications for the "material-first" scenario and align wood flow quality criteria with the requirements of each application. With this, we can combine LCA and MFA and create decision support for optimal cascading use, ensuring the most efficient and sustainable use of wood resources as carbon sinks and storage in the technosphere and a mature biomass strategy of whether a flow could be used to introduce a new material application, a recycling or reuse cycle, or for energy production. Our work advances scientific understanding and offers real-world solutions for sustainable wood use and carbon mitigation.

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5.07.P-Tu437 Holistic Environmental Assessment of Packaging and Potential Material Substitution Impacts

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To properly evaluate the potential life cycle environmental impacts of packaging materials, it is essential to consider a broad range of environmental factors. A thorough, science-driven assessment should aim to account for each stage of a packaging life cycle, from material production to end-of-life disposal, ensuring a holistic and well-rounded analysis of its potential overall environmental impact.

In a comprehensive Life Cycle Assessment (LCA) study according to ISO 14040 and 14044 the potential life cycle environmental impacts of 41 distinct packaging formats sampled from European supermarkets were assessed to compare polyethylene-based packaging with alternative non-plastic materials using PEF (Product Environmental Footprint) methodology for the transport and End of Life (EoL) analysis. The use phase (e.g., product loss, breakage rates) was excluded from the assessment. The following polyethylene product categories were covered:

- Collation shrink packaging (2 packaged products and 5 packaging formats)
- Heavy-duty sacks (2 packaged products and 4 packaging formats)
- Flexible food packaging (8 packaged products and 20 packaging formats)
- Pallet wrap (1 packaged product and 3 packaging formats)
- Rigid non-food packaging (4 packaged products and 9 packaging formats)

It can be shown that in a number of comparisons the substitution of PE-based packaging could lead to

higher GWP-emissions. For the other impact categories considered, the results showed generally lower potential impacts for PE-based packaging (acidification, eutrophication freshwater, water use, land use), and for fossil resource use no clear trend was discerned. The study showed that banning or restricting the use of plastics such as polyethylene for food packaging could lead to higher greenhouse gas emissions for many applications. Thus, selection of the packaging formats to enable the lowest overall environmental impact is a complex process that requires comprehensive analysis.

5.08 Life Cycle Impact Assessment Modeling Including Normalization & Weighting

5.08.T-01 Getting Water Impacts Right - Methodological Developments from Inventory Modelling to Regionalized Impact Assessment in the ecoinvent Database

Thomas Sonderegger and Guillaume Bourgault, ecoinvent, Switzerland

The use of regionalized impact assessment methods in software and background databases is still limited. This work shows how improving water modelling and balancing, increasing regional coverage, and using geospatial data in application of regionalized LCIA methods play together for improved and regionalized water impact assessment in the ecoinvent database.

Based on discussions with ecoinvent-internal and external experts, we developed a new water balancing model for biomass growth including new flows and properties. Improved water balancing improves the reliability of amounts of water flows in inventories, which are assessed later.

Regional differences start with inventories where different ways of production can result in, for example, different water amounts to be characterized in impact assessment. Therefore, ecoinvent strives to cover more regions with regionally specific inventories. For the case of water, it is important to align global coverage where water is passed from one process to another. For example, each region with irrigated crop production will get an irrigation process specific for that region.

Currently, some software tools use regionalized elementary flows to implement regionalized methods. Brightway2 does so using geospatial data. However, there was no extensive and consistent application of geospatial data to the ecoinvent database yet. Maps for crop production, for example, allow connecting inventories to native spatial scales of methods such as AWARE for water impacts: we can compute how much crop is produced in which watershed within a region, which allows calculating regionally weighted characterization factors. First results from applying these characterization factors show that the suggested approach can be implemented on a background database. Comparing regionalized results to those using a global characterization factor show changes to be as expected, which confirms that the approach works and improves water impact assessment.

ecoinvent's efforts in improving and regionalizing inventories as well as the approach of using geospatial data improve water impact results. This will offer regionalized impact assessment scores to ecoinvent users and possibilities for software and tool developers to use background LCA databases for regionalization in new ways. All this helps strengthening regionalization efforts in the LCA community.

5.08.T-02 Midpoint Characterization Model for Water Consumption Impacts on Aquatic Ecosystem: RESCUE Model

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The impacts of water consumption through life cycle of products/services/organizations have been the subject of considerable debates. The AWARE model has been widely used for the assessment of the impacts of water consumption as a generic midpoint indicator, while demand for ecosystem-specific characterization models for water consumption has been increasing with the growth of request and interest in sustainable management of ecosystems along with the international initiatives, e.g. IPBES, Taskforce on Nature-related Financial Disclosures, and Nature Positive Initiative. In response to this demand, we have developed a new midpoint characterization model for assessing the impacts of water consumption on aquatic ecosystems: RESCUE model. The developed ecosystem specific model has some similarity with AWARE model in the modeling concept, while it specifically captures the potential impacts on aquatic ecosystems. First, environmental water requirement for aquatic ecosystem is defined based on the flow regimes in a watershed, and then, overconsumption of water by human activities is determined to assess the extent of deprivation of water for aquatic ecosystem as a potential impact on ecosystems. We have developed characterization factors for around 11,000 watersheds covering the whole globe which can be also aggregated into country scale. As a result, 67% of the global watersheds where we consume water already face the deprivation of water for aquatic ecosystem. The extent of deprivation reaches at 60% of

water for aquatic ecosystem requirement. In this presentation, we present the details of the model and results, and highlight the similarity and differences between RESCUE and AWARE model.

5.08.T-03 Accounting for Specific Impacts of Fisheries in the Environmental Footprint Method – Combining Fishery Science and LCA

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The Product Environmental Footprint (PEF) has been designed by the European Commission (EC) to assess and disclose environmental footprints of all consumer goods in Europe. However, when it comes to fishery products, indicators related to i) (the living) resource exploitation and ii) seabed impacts from fishing gears such as bottom trawls are lacking, despite being major drivers of environmental impacts from fishing.

The EC has asked four experts (two LCA experts and two fishery scientists) to provide recommendations on how to address those impacts in the Environmental Footprint (EF) method. The experts first completed a review of methods to assess environmental impacts of fishing on i) resources and ii) seabed impact. Each expert was assigned one of four domains, defined as a combination of i) an impact pathway (resource or seabed) and ii) a science realm (LCA or fishery science); each completed the review, and recommended a single, most up to date method in his/her assigned domain, resulting overall in 4 recommended methods. The experts then discussed the possibility of incorporating each of the 4 recommended methods into EF, adding two indicators to the current 16 indicators. They discussed if and how normalization was feasible, and they applied the methods to three fishing case studies at landing (Hake from the Bay of Biscay, fished with i) bottom trawl (case study 1) ii) midwater trawl (case study 2) and iii) Argentinian Red Shrimp fished with a pair of beam trawls (case study 3). Based on the results, they proposed a roadmap for accounting for those impact pathways in EF.

The two fishery-science-based methods have been developed by the Scientific, Technical and Economic Committee on Fisheries (STECF) of the EC; they are natively qualitative and non-additive. Proposals were made to relate qualitative and quantitative scores, and normalization factors were computed. As the CFs for the seabed-impact recommended LCA method were only available at European level, it was not possible to compute normalization factors.

Regarding impact on resources, results of case studies displayed a variability of several orders of magnitude between i) case studies 1 and 2 and ii) case study 3 for the LCA method, and a variability lower than one order of magnitude for the STECF method. Regarding seabed impact, results from the LCA method could not be computed for case study 3; the STECF method displayed a variability of a factor lower than an order of magnitude.

Disclaimer/Disclosure: The views expressed in the article are personal and do not reflect an official position of the European Commission.

5.08.T-04 Cellulosic Versus Synthetic Fibers: How Do They Compare in a Life Cycle Assessment When Fibers Emissions are Included?

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Textile clothing is a significant contributor to microfiber pollution in marine ecosystems. A significant portion of microfibers emitted are cellulosic-based, either natural (e.g. cotton and linen) or regenerated cellulose (e.g. viscose and lyocell). When emitted, these particles are readily ingested by marine organisms, posing harmful effects even at environmentally relevant concentrations.

In Life Cycle Impacts Assessment (LCIA), characterization factors (CFs) have been established for synthetic microfibers. However, no such CFs exist for cellulosic microfibers (CMFs) due to the lack of exposure and effect factor (EEF) specific to these fibers. Current LCIA methods therefore overlook potential environmental impacts from CMFs. Given that natural and regenerated cellulosic textiles release more microfibers when washed and are found in greater abundance in the environment than synthetic microfibers, CFs specific to these fibers are an important gap that this work aims at addressing.

The fate of CMFs in the marine environment combines the fate in the water column and sediments, differentiating between different CMFs materials and sizes. Further, an EEF for CMFs is developed. The EEF is calculated from a hazardous concentration of 20% (HC20), derived from a species sensitivity distribution (SSD) of effect concentrations of 10% (EC10). The EEF was extrapolated to sediments and the fate was combined with EEFs to obtain midpoint and endpoint CFs that include impacts in water and sediments. The CFs were then tested in a textile LCA case study, comparing the cradle-to-grave ecosystem quality impacts of a cotton t-shirt and a polyester (PES) t-shirt.

The EEF obtained is 2950 PAF.m³/kg, which is not significantly different from that of MPs. Marine midpoint and endpoint CFs for 6 cellulosic materials (cotton, linen, viscose, rayon, lyocell and modal) of 5 different sizes (1 to 5000 µm) and their uncertainty were computed. For the same size, the CFs of CMFs are on average one to two orders of magnitude smaller than the CFs of synthetic microfibers, largely due to their smaller residence time (due to faster degradation) in the marine environment. In the textile case study, the impacts of PES emissions were found to be a significant contributor to EQ damage, but not in the cotton t-shirt case. Multiple sensitivity analyses on the use location, end-of-life scenario and release rates are conducted. CFs for CMFs are readily available for environmental decision-making.

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5.08.T-05 Life Cycle Impact Assessment for Positive and Negative Impacts of Offshore Wind Farms on Benthic Marine Biodiversity

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The construction and decommissioning of offshore wind farms affect the marine ecosystem by changing the marine habitat. Construction can occur in different substrate types, and decommissioning can follow scenarios varying from complete removal to leaving parts of the structure in the sea, and each scenario will impact marine biodiversity differently. But how to make informed decisions when quantitative assessment methods representing the marine biodiversity impacts of marine habitat changes are lacking?

In this study, we develop Characterization Factors (CFs) for life cycle impact assessment considering the benefits and impacts of offshore wind farm construction and decommissioning on marine benthic biodiversity. The CFs are disaggregated for different taxonomic groups and alien species to allow for assessment of the species composition.

We use a dataset that includes data on benthic species richness for 17 offshore structures and natural seabed samples in the North Sea. The species identified in the dataset are divided into 8 different taxonomic groups based on their phylum, and alien species are determined by comparing the species to the IUCN list of alien species in the North Sea. For each taxonomic group and alien species, we developed polynomial models expressing the species richness on the structure as a function of structure age, seabed type, and decommissioning scenario. The richness models are used to develop CFs expressing the locally disappeared or gained fraction of species associated with each construction or decommissioning scenario, integrated over the considered timeframe (PDF.year).

The species richness on the structures generally increases during the structure lifetime, but the CFs show that construction on soft seabed generally results in a net species loss, while construction on hard seabed results in a net gain of species. All decommissioning scenarios result in a loss in the total number of species, but the CFs indicate that leaving parts of the structure will preserve 80-99% of the richness. Additionally, the CFs indicate that construction and decommissioning activities will cause considerable changes in the richness of alien species and shifts in richness between taxonomic groups. The developed CFs provide a basis for understanding and managing the ecological consequences of offshore wind projects, considering both positive and negative impacts and the changes in species composition.

5.08.P Life Cycle Impact Assessment Modeling Including Normalization & Weighting

5.08.P-We421 Influence of Different Normalization and Weighting Methods in Life Cycle Impact Assessment: Single-Score Assessments of a District Cooling Plant

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District cooling plants are becoming increasingly important as they play a key role in providing energy efficient and sustainable urban cooling solutions when climate change drives temperature increases globally. Single-score results in LCIA can enable effective communication and decision making. The aim of this study is to investigate the effects of different normalization and weighting methods, applied to the case study of the district cooling plant "Wien Schottenring," in Vienna, Austria.

The plant has a cooling capacity of 19.8 MW and operates a combination of three compression chillers and two absorption chillers. The compression chillers are powered by grid electricity, while the absorption chillers use predominantly district heat as their energy source. A time-resolved modelling approach is used to analyze electricity and district heat supplies as well as the cooling demand, taking into account time variations in energy flows and consumption. LCA results will be compared for two cooling variants:

Variant 1 (existing configuration): Cooling with both absorption and compression chillers.

Variant 2 (alternative configuration): Cooling with compression chillers only.

Various normalization and weighting methods will be applied to arrive at single-score LCIA results. These include ReCiPe, EF 3.1, the ecological scarcity method, and alternative normalization approaches from recent literature. Finally, a normalization based on national Austrian data will be applied to account for regional specificities.

The robustness of the comparison between the two variants will be evaluated with regard to the choice of normalization and weighting approaches and to the resulting single-score results. Also, the contributions of individual categories to the single score will be analyzed, both for uniformly weighted normalization methods and for weighting methods (ReCiPe and EF). Finally, we will show the effect of applying a regionalized Austrian normalization set compared to the global and EU-wide normalization sets.

The study will apply a range of normalization and weighting methods to one of the future key technologies for urban climate change adaptation. Single-score LCA results can easily be communicated to non-experts, making a careful analysis of the underlying methods critical to providing robust decision support for industry and policymakers.

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5.08.P-We422 Simplified Environmental Scores for Greener Vehicle Procurement: Evaluation of Weighting Effects in Belgium's Ecoscore

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Life cycle assessment (LCA) is a great tool to enhance more sustainable procurements of vehicles. However, it can be quite complex for non LCA-experts. It is why, in several countries, environmental rating tools for vehicles emerge as they are easier to understand, using a simple, comprehensible value. In Belgium, Ecoscore were developed in 2003, providing a single score for each car in the Belgium fleet and used both by consumers and policymakers. The creation of such scores typically involves normalization and weighting steps, which can introduce uncertainties. The goal of the study is, for the specific case of the Belgium score, to assess how Ecoscore can balance between simplified results communication and accuracy of the underlying data.

The Ecoscore method is currently being updated from a well-to-wheel approach to LCA method. The normalization step has been updated by changing the reference vehicle to an electric vehicle with a 27.8 kWh battery. In addition, the Environmental Footprint 3.1 (EF3.1) methodology has been adapted to include only emissions already accounted for in the original Ecoscore, as results from homologation tests are available. These emissions are classified into the corresponding impact categories. The weightings have been adapted from EF3.1.

To meet the objective, it is important to understand how the weighting of different environmental categories influences the Belgium score. The study will be carried out first, at vehicle level, where the weightings will be modelled as parameters in the LCA. A one at a time parameter modification is conducted to compare the final scores while keeping all other parameters constant. Secondly, at fleet level, the single score will be evaluated for the Belgian fleet and compared to results conducting LCA using PEF weightings and including all emissions to identify the main differences and similarities between the two methodologies.

The results will show the scores calculated at vehicle and fleet level for the different assessment method. It should provide valuable insight into whether the Belgium score is representative of the actual environmental impact of the car fleet being rated. In the context of greener procurement, it's crucial to understand how the trade-offs between simplification for non-experts and the scientific accuracy might impact the effectiveness of the score in both guiding consumers and supporting policy goals for sustainability.

5.08.P-We423 ParaBAT: A Methodology of Progressive Approaches to Improving Systems Aimed at Minimizing Waste Production Originating From Industrial Activities

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Achieving the best available techniques in the operation of large industrial and agricultural facilities represents one of the most important tools for environmental protection. Reaching emission limits, or even the lower boundary of BAT (Best Available Techniques) emission limits, usually requires the introduction of new technology or modification of existing technology, which entails additional material or energy costs. The acquisition or production of these material or energy inputs typically involves technological processes that, through their operation, may release varying amounts of emissions into different environmental compartments. The aim of the ParaBAT methodology is to enable the evaluation of the actual benefit of reducing emission limits throughout the entire supply chain. Under suitable conditions, the methodology can be used to justify why the lower, stricter boundary of the BAT emission limit is not met by the evaluated technology. The ParaBAT methodology appropriately applies the LCA (Life Cycle Assessment) method and specifies its use specifically for the purposes of evaluating the achievement of BAT goals. The novelty of the methodology lies in defining a precise and clear procedure for evaluating environmental impacts using the PEF (Product Environmental Footprint) method in the Czech Republic, with a focus on technologies for the production of secondary raw materials and recyclates. The methodology describes the framework and basic procedure for assessing the environmental impacts of BAT technologies, including the accounting of input materials and energy, and the output of by-products, thus also accounting for indirect emissions from the operation of the evaluated technology.

5.08.P-We424 Characterization of Land Use Impacts on Evaporation-Precipitation Dynamics and Its Role in Earth System Functioning

Jan Matušík, Charles University Environment Centre, Czech Republic

Water plays multiple roles in the Earth system functioning and stability, and all the water sub-systems are currently affected by human activity. As one of those issues, change in evapo-transpiration caused by land use change can have a significant impact on rainfall and water availability, yet this issue is not well covered by available Life Cycle Impact Assessment methods. This study aims to model land use effects on evaporation dynamics through the lens of Earth system stability. This differs from available methods which mostly frame this issue in the context of green water flows and their effect on local soil water availability. The proposed characterization method quantifies the change in potential evapo-transpiration in comparison to a respective reference. For a study on a small scale, such as product-level, the impact is quantified in absolute values - regardless of whether the flows increase or decrease, it is a potentially adverse change from the reference stable conditions. To capture the relative importance of land use for terrestrial water availability, this change is further weighted by continental evaporation recycling ratio which measures how much of the evaporation returns to land as precipitation. Since both parameters are highly location- and season-specific, regionalization on as fine resolution as possible is advisable. However, aggregated characterization on a country and a year level seems the most practical. Next to land cover, evaporation dynamics are strongly influenced by climate and its changes. Thus, in addition to the baseline model, prospective characterization factors accounting for the effect of climate change should be developed. While the issue of evaporation-precipitation dynamics does not immediately appear as the most pressing environmental issue, the impact on water availability can be considerable in some locations dependent on moisture recycling, such as the Amazon. Hence, being able to evaluate potential impacts of products on this element of the Earth-system can allow to consider further dimensions of life-cycle environmental consequences of production systems.

5.08.P-We425 Advancing Soil Quality Integration in LCA

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Healthy soils are in good chemical, biological and physical condition so that they can provide ecosystem services. The Land Footprint (LAFO) model has recently been developed to estimate the EU's yearly land footprint but a geospatial component could be added to the existing model to link the land footprint information to soil characteristics. The Soil Use Calculator is a novel tool developed to estimate the environmental impacts of crop production on soil health. The calculator integrates spatial data on land use, soil type, crop requirements, soil properties, and soil erosion to assess the soil footprint of various crops. This study presents the development and application of the calculator, focusing on the impact of soil organic carbon levels on global soybean production. The calculator uses a methodological approach that considers the land area required to produce crops, soil degradation rates based on soil properties, and soil indicator thresholds. The results from LAFO model show that soybeans are the most widely imported crop

in the EU, mainly sourced from Argentina and Brazil. The global cultivated area with soybean was distributed across four levels of Soil Organic Carbon (SOC) constraints: no constraint (49%), slight constraint (37%), moderate (11%), and severe_non suitable (4%). The proposed methodology for a soil footprint calculator provides a framework for estimating the soil-related environmental impacts of crop production. The soil use calculator can be used to identify areas with high soil degradation potential and to prioritize conservation efforts, and can be integrated into EF to get a more precise environmental impact assessment by improving the baseline. Further research is needed to refine the calculator and to apply it to other crops and by using other soil characteristics, as those present in the EF Soil quality index used in impact assessment.

5.08.P-We426 Including Environmentally Relevant Effects of Microplastics in Life Cycle Impact Assessment

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Plastics pollution occurs on a large scale and has become a severe environmental problem for all different ecosystems across the globe. Multiple studies have worked on incorporating micro- and macroplastics into Life Cycle Impact Assessment (LCIA). The derived effect factors (EF) in these studies are based on Species Sensitivity Distributions (SSDs) fitted to mass-based effect concentrations of often monodisperse microplastic particles. In reality, however, microplastics that occur in the environment span a wide range of shapes and sizes. As a result, the SSDs and consequently the derived EFs do not accurately represent the effects of polydisperse microplastics in the environment.

Therefore, in this study we aimed to incorporate environmentally relevant effects of microplastics on terrestrial and aquatic ecosystems in ReCiPe a multi-impact LCIA methodology. For this new EF were derived for the terrestrial and aquatic environment based on SSD that were corrected for the polydispersity of environmental microplastics and for the bioaccessible fraction. These SSDs are also used in environmental risk assessment to assess the risk of microplastics to the environment more accurately. Furthermore, fate factors for 13 polymers and for each receiving compartment were derived using an adapted version of SimpleBox4Plastics. For this, adjustments were made to be able to model the fate of plastics from a mass-based inventory flow to size-based microplastic concentrations in the receiving compartments. Subsequently, fate and effect factors were combined to derive characterization factors for microplastics in the environment.

In the presentation, we will explain the process developing the EF and FF for microplastics. Hereby, emphasizing the importance of correcting laboratory-based effect concentration for bioaccessibility and the alignment of these laboratory-based concentrations with environmentally realistic microplastic size ranges. Furthermore, we will present characterization factors for terrestrial and aquatic ecosystems. We will also use a case study to compare the developed characterization factors with earlier LCIA studies on microplastics based on a case study. Overall, the presented work increases the accuracy of LCIA for microplastics and provides tools to better evaluate the environmental impacts of plastics.

5.08.P-We427 Exploring Global Supply Chain Structures for Sustainable Consumption of Freshwater at Global and Regional Scales

Keitaro Maeno, Masaharu Motoshita and Kamrul Islam, National Institute of Advanced Industrial Science and Technology, Japan

The expansion of global supply chains (GSCs) of industries has led to the concentration of demands for freshwater resources in some specific regions, causing excessive pressure on local human health and ecosystems in those regions with overconsumption of freshwater. On the other hand, industrial production activities in each country involved in the GSCs bring not only such environmental interventions (i.e., negative effects) but also economic benefits (i.e., positive effects) on the local economy. However, a relationship between these opposite effects of the GSCs and GSC structures which can balance them still remain unclear in the existing literatures. In this study, we explore the sustainable GSC structures aiming at reducing water overconsumption in each country included in specific GSCs while considering the balance of the positive and negative effects at both global and regional scales. Through this process, we estimated global water overconsumption data in 2015 based on WaterGAP 2.2d model and integrated it into a multi-regional input-output (MRIO) framework. This approach allowed us to assess water overconsumption in each country involved in specific GSCs and compare it with economic value-added simultaneously generated in those country. The results focusing on the GSCs of EU s, USA s, Chinese industries revealed that India experienced the largest volume of water overconsumption, accounting for

more than 25% of the total water overconsumption induced in the relevant GSCs. This result means that industries in EU, USA, China impose significant pressure on local human living and ecosystems in India through their GSCs. Conversely, we found relatively larger value-added was created by the relevant GSCs in India with respect to the volume of water overconsumption, compared to that in several African countries such as Ethiopia and Kenya. Based on these findings, we highlighted that there are large gaps between the positive and negative effects among country within the relevant GSCs, and implicated a pathway for the relevant industries to mitigate the gaps by transitioning their GSCs toward structures that balance these effects in each country.

5.08.P-We428 Improving the Accountability of Green Water Flows and Stocks in Agricultural Life Cycle Assessment

Tamara Schmidt and Montserrat Nunez, IRTA, Spain

Green water, defined as soil-stored rainwater consumed by plants through evapotranspiration (ET), is a crucial component of agricultural water use. However, it remains underrepresented in Life Cycle Assessment (LCA) frameworks, which predominantly focus on blue water (surface and groundwater). Given that agriculture accounts for ~70% of global water use and ~90% of consumption primarily through ET this omission limits the capacity to fully assess agricultural systems' environmental impacts. Current approaches to green water accounting, such as LANCA or net green water consumption, provide implicit assessments based on land use but fail to quantify environmental impacts in terms of ecosystem quality. This hinders the correct integration and impacts of green and blue water flows in LCA.

This study addresses this gap by enhancing LCA frameworks to explicitly account for green water. Building on the mechanistic framework for water use impact assessment, we employ SWAT+ (Soil and Water Assessment Tool) to model water stocks and flows in Catalonia, Spain. SWAT+ was calibrated to high-resolution datasets (20x20 m, 2013-2023) and validated against satellite-derived ET data (2019-2023). Using a hydrological model enables the unique derivation of important hydrological variables and crop-specific water demands at the same time.

Initial outputs highlight green water's significant role in water availability and flows across interconnected environmental compartments. By incorporating inter-annual and intra-annual crop rotations for major crops represented in augmented land-use maps, we provide more accurate results of modelled ET. At the Life Cycle Inventory (LCI) level, these results provide the basis to generate a database of average field water flow data. At the Life Cycle Impact Assessment (LCIA) level, hydrological outputs from SWAT+ enable the development of fate factors, quantifying how green water consumption affects soil moisture, aquifer recharge, and river baseflows.

Our approach emphasizes the need for accurate land-use data and calibrated models to reliably assess water consumption's environmental impacts. These insights support policies prioritizing sustainable water management, particularly in regions like the Mediterranean, where both green and blue water are critical for agriculture.

5.08.P-We429 Development of Freshwater Ecotoxicity Characterization Factors based on Nonlinear concentration-response function

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Of the nine planetary boundaries identified as critical thresholds for Earth's stability, six have already been exceeded. These include climate change, novel entities, and loss of biosphere integrity. This highlights the urgent need to assess chemical risks to ecosystems. Life cycle assessment (LCA) methodologies have been developed to address these risks at the global and national levels; prominent LCA models such as LC-IMPACT, IMPACT World+, and ReCiPe 2016 calculate characterization factors (CFs) for ecotoxicity. On the other hand, LC-IMPACT and IMPACT World+ rely on chronic EC50 data and USEtox models that use a 0.5/HC50 approach; HC50 (Hazard Concentration 50%) is derived from the EC50 value. More recently, CF has been calculated using 0.2/HC20, adopting an index closer to the environmental concentration, such as EC10. However, CF calculation methods remain linear; Tang et al. (2023) showed that nonlinear CFs based on toxic mode of action (TMoA) can improve the accuracy of distinguishing chemical effects. These refined coefficients can help identify priority chemicals for risk reduction but require more input data.

This study develops non-linear CFs on a global scale using the latest LCI (global chemical emissions) data. By integrating non-linear CFs with global LCI data, we aim to calculate the Normalization Value for freshwater ecotoxicity at the global level. Additionally, we compare these results with those derived using linear CFs to assess methodological differences.

The LCI data included 690 chemicals, but the number of chemicals was reduced to 505 because of the

combination with the USEtox model. Moreover, we used toxicity data from reliable data sources and only results with more than five species were used, resulting in characterization factors for 196 substances. Using the above methods, we analyzed global ecotoxicity results and attempted to identify trends in which substances are currently having the greatest impact. We compared CFs with those calculated by existing global models and compared the differences in toxicity mechanisms and HC20 toxicity data used in the Environmental Footprint, leading to future risk assessment methods with chemical substances. We will expand the range of target substances by using other toxicity test data and extrapolation methods.

5.08.P-We430 Basin-Specific Fate Factors for Freshwater Ecotoxicity of Pharmaceuticals

Tolga Ayeri¹, Selwyn Hoeks¹, Rosalie van Zelm¹ and Ad Ragas¹, (1)Radboud University, Netherlands
Pharmaceuticals play a crucial role in protecting and improving human health. However, they can persist in freshwater for extended periods, ranging from months to years, since conventional wastewater treatment plants (WWTPs) may not fully remove them. Therefore, understanding their persistence in water is essential to assess the potential environmental impacts of pharmaceutical residues on aquatic ecosystems.

Life cycle assessment (LCA) is a widely used method to quantify the environmental impacts of a product or service. In the Life Cycle Impact Assessment (LCIA) step, the magnitude and importance of the potential environmental impacts of a product system are quantified. For this, characterization Factors (CFs) are used to transform emissions and resource extractions into environmental impact scores on various impact categories, such as ecotoxicity. CFs for ecotoxicity include a fate factor (FF), which describes the change in the steady-state mass of a chemical resulting from a change in emission flow. Currently, FFs for freshwater ecotoxicity are country- or continent-specific, neglecting region-specific effects. Since pharmaceuticals are sensitive to variations in local water conditions and their emissions originating from sewage systems are site-specific, there is a need to derive basin-specific FFs for freshwater ecotoxicity. Thus, this study aimed to derive basin-specific FFs for pharmaceutical emissions into freshwater across Europe at 1 km resolution. As a proof of concept, FFs were derived for the river Ouse (UK) and Rhine basins (North West Europe) as examples. To extend this analysis, additional results for a broader range of pharmaceuticals across all European river basins will be presented during the poster or presentation.

Results showed that the FF in the river Rhine basin was approximately four times higher than the river Ouse basin, due to the larger size of the river Rhine basin. Longer river lengths within a basin result in increased retention times, allowing diclofenac to persist longer in the system. However, the generic FF for diclofenac in USEtox, a scientific consensus model to derive CFs for freshwater ecotoxicity, is 39.60 days. This value is approximately 20 times higher than the FF for the Rhine basin. These findings highlight the importance of accounting for the spatial heterogeneity of pharmaceutical emissions to freshwater within LCIA, ultimately enhancing LCA studies in healthcare.

5.08.P-We431 Clustering Methodology Developed by the EcoBeautyScore Association to Improve the Coverage of Freshwater Ecotoxicity Characterization Factors of Cosmetic Ingredients

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The EcoBeautyScore (EBS) Association*, comprising over 70 global cosmetic companies and associations, has developed a unified environmental footprint measurement and scoring system for cosmetic products over the past three years. This system is based on Life Cycle Assessment (LCA), recognized by the European Commission (EC) as the most effective method for assessing and comparing the environmental footprint of products and services. The LCA methodology aligns with the Product Environmental Footprint (PEF) guideline initiated by the EC in 2013. Moreover, the method was enhanced to suit the specificities of the cosmetic industry.

The Association members recognized a need to develop more comprehensive databases to improve coverage for environmental characteristics of cosmetic ingredients to make PEF applicable to cosmetic products. This need is driven by the large portfolio of diverse ingredients used in cosmetic product formulation, and particularly for mid-point characterization factors (CFs) to assess end-of-life ingredient impacts, which is a significant differentiator among EBS results for cosmetic products.

The CF is the product of fate (FF), exposure (XF) and effect factor (EF) values. The Association concentrated efforts on developing substance-specific EFs because they are the most influential factor for determining CF, described in a separate presentation. The FF and XF values are data intensive to derive, needing a minimum of five physical/chemical and four environmental characteristics. Because of the large ingredient portfolio used in cosmetic products and the smaller relative influence of FF and XF, a semi-specific approach to determine FF and XF was developed using a read across and clustering method. Semi-specific FFs and XFs, and specific and semi-specific EFs were combined to create a robust CF database for cosmetic ingredient end-of-life assessment.

We created a matrix of four semi specific clusters for the product of XF and FF values, based on ingredient biodegradability and bioaccumulation properties. This generated data for 30% of the Association's priority ingredients when combined with substance-specific EF values. When no such EF value was available, four semi-specific EFs were used to obtain 16 clusters, enabling CF estimation for additional priority ingredients. This approach produced a significant improvement in data coverage for ingredients contributing most to the end-of-life environmental footprint of cosmetic products.

Disclaimer/Disclosure: The EcoBeautyScore initiative started as a consortium in 2021 and turned into an association (under the Belgium Law) in 2024.

5.08.P-We432 How the Effect Factor Calculation Method Developed by the EcoBeautyScore Association Improves the Robustness of Freshwater Ecotoxicity Impact Assessment of Cosmetic Products in Life Cycle Assessment

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The EcoBeautyScore (EBS) Association*, comprising over 70 global cosmetic companies and professional associations, has developed a unified environmental footprint measurement and scoring system for cosmetic products over the past three years. This system is based on Life Cycle Assessment (LCA), recognized by the European Commission (EC) as the most effective method for assessing and comparing the environmental footprint of products and services. The LCA methodology builds on the Product Environmental Footprint (PEF) guideline initiated by the EC in 2013. Moreover, the method was enhanced to suit the specificities of cosmetics. One of the key topics subject to adjustments from PEF in the EBS methodology is the freshwater ecotoxicity impact assessment method.

For freshwater ecotoxicity, the PEF method prescribes the use of the USEtox© 2.1 model adapted by the Joint Research Center (EC). There is a consensus within the EBS Association that the prescribed USEtox model for freshwater ecotoxicity and the corresponding characterization factors (CF) (as part of the EF 3.1 method package) have limitations in their ability to generate robust environmental scores for cosmetics, suitable for meaningful differentiation among products, which is the main objective of the Association. The actions taken within the EBS Association to tackle these limitations and improve the robustness of the freshwater ecotoxicity CF led to the development of an alternative method, called MSS HC5, to calculate the Effect Factor (EF) component of the CF value. The method calculates the EF component of CF using the 10% response levels in chronic aquatic toxicity tests (EC10chr, used preferentially) or no adverse effects concentrations (NOECs) of the Most Sensitive Species (MSS) determined from available chronic studies or extrapolated from acute toxicity studies, if necessary. Data for the three standard trophic levels (algae, invertebrates and fish), are used to approximate the Hazard Concentration for 5% of all freshwater species (HC5). This alternative method, which sensitivity analyses indicate is equally or more robust than the PEF-prescribed method using an HC20 derived from a Species Sensitivity Distribution (SSD), are shown, as well as its advantages for the cosmetics sector and potentially other sectors motivated to improve reliability of freshwater ecotoxicity impact assessment in LCA.

Disclaimer/Disclosure: The EcoBeautyScore initiative started as a consortium in 2021 and turned into an association (under the Belgium Law) in 2024.

5.08.P-We433 Applying Product Biodiversity Footprint Method To Cosmetic Sector: Case Study Of L'Oréal

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As biodiversity declines globally, it is essential to assess the biodiversity footprint of individual products, industrial sites, and at the company level. There are many different approaches in use, but no consensus exists on which should be endorsed.

I Care has been working since 2017 on developing a suitable methodology to assess the impact on biodiversity of products. L'Oréal participated in the development of this methodology, called Product Biodiversity Footprint (PBF), and was associated with Sayari in its publication in 2019. It is essential for L'Oréal to develop an understanding of the biodiversity footprint at product level to support eco-design of cosmetics with the aim of preserving or even restoring biodiversity, as well as exploring how it connects to corporate commitments..

In 2024, the PBF methodology underwent significant changes by incorporating Mean Species Abundance metrics to assess biodiversity loss. Based on the 2019 Global Assessment report from the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, a new approach for weighting biodiversity pressures was introduced.

The PBF methodology allows to evaluate the impact on biodiversity of different practices and actions introduced at the product level, by comparing a reference product to variants of this product. Three cosmetic products were studied to demonstrate the interest of the revised methodology: a shampoo, a shower gel, and a deodorant. The analysis was also conducted at the ingredient level (glycerin, sodium laureth sulfate, cocamidopropyl betaine, ammonium lauryl sulfate and ethanol) and agricultural feedstock level (palm crop, coconut crop, soybean crop and beetroot crop) to better understand biodiversity impacts. The results are encouraging in guiding future choices among various alternatives of cosmetic products under development, also taking their biodiversity footprints into account.

5.08.P-We434 Development of Extinction Risk Maps for Terrestrial Species Due to Climate Change

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According to IPBES report, the impact of climate change on biodiversity is extremely severe, and an effective tool is required to address the ongoing loss of biodiversity. Specifically, it is necessary to quantitatively evaluate the impacts from climate change on biodiversity worldwide and to assess the extinction risk of species in the future at a high spatial resolution.

Recently, a study estimated spatially on the impacts of climate change to biodiversity in each taxonomy group. This study used the Potentially Affected Fraction (PAF) as an indicator to estimate the impacts, without directly addressing potential extinction risks. Additionally, the TNFD guidelines emphasized the importance of evaluating regional biodiversity loss with higher resolution. On the other hand, in LCIA, little progress has been made in developing biodiversity damage factors at the grid level to reflect spatial differences caused by climate change. Given that biodiversity and environmental factors vary regionally, it is essential to evaluate the potential extinction risks of species at the grid level. Such evaluations enable a more accurate understanding of the extent to which specific regions and species are at risk due to climate change, forming the basis for developing appropriate conservation strategies.

Therefore, this study aims to examine how future climate change scenarios will affect the extinction risk of terrestrial species and to estimate the distribution of global extinction risks and climate change-induced damage factors at the grid level.

Specifically, species distribution model (SDM) was used to evaluate a total of 8,428 terrestrial species across five taxonomic groups. Habitat changes and losses due to climate change were simulated under SSP3-RCP7.0, a sustainable future scenario, and extinction risks were estimated from changes in the species' habitat areas. The estimated extinction risks were then allocated to grid levels to illustrate the spatial distribution of extinction risks. Through this approach, the study aims to identify regions in urgent need of conservation measures and contribute to the development of future biodiversity conservation strategies.

5.08.P-We435 Location-Based Global Biodiversity Extinction Damage Factor Development—Under Future Land Use Change Impact

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Following the establishment of the Kunming-Montreal Biodiversity Framework, there has been a significant global trend in the assessment of ecosystems and biodiversity. Nevertheless, the current tools and knowledge for evaluating the impact from land use to biodiversity still demonstrate significant gaps that require enhancement. Advancements in assessment methodologies and precision will further support the realization of global Natural-Positive objectives.

We have significantly optimized the existing methodologies utilized for assessing biodiversity, enhancing their accuracy to ensure that users of Life Cycle Assessment (LCA) can perform evaluations that are specific to particular locations in future applications. In this research, we have further expanded the coverage scale to encompass an impressive total of 400,000 species across five distinct taxa. These findings build upon our previous work, which concentrated on the evaluation of 6,000 species and future predictions based on land use data characterized by the CIMP6. Conducting assessments at the species level necessitated the integration of a machine learning model that incorporates detailed species-level information. The results of our analysis extend from the present day to the year 2100, under various future scenarios. In contrast to earlier research, we have made an effort to introduce the extinction rate indicator derived from the Planetary Boundaries report, integrating it into the life cycle impact assessment model. This innovative approach not only allows for a more comprehensive interpretation of the assessments but also facilitates the evaluation process to align with policy-oriented standards. To address the anticipated demands of future users, we have accurately segmented the damage factor on both a high-resolution spatial scale and a country-specific numerical scale for certain regions, even refining these scales to a prefectural level in some instances.

5.08.P-We437 Use of Satellite Data and Spatially Differentiated Characterization Factors for Biodiversity Impact of Land Use Due to Wind Turbines

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The impact of wind energy deployment on biodiversity mostly originates from habitat loss and disturbance. The assessment of the impact of land use (LU) on biodiversity necessitates spatial differentiation. To be able to assess the LU impact of wind turbines (WT), detection of LU change thanks to satellite data is necessary. However, there is a mismatch between the LU types in life cycle impact assessment (LCIA) and satellite data. In order to assess the European WT fleet, a model taking into account spatial explicit characterization factors (CF), satellite data and WT parameters is needed. Our model needs the WT location, capacity, hub height and rotor diameter as user inputs. Corinne Land Cover 2018 100mx100m dataset is consulted to evaluate pre-installation LU type. For each ecoregion of Europe, land use type from Corinne dataset and land use type and intensity data from life cycle impact assessment (LCIA) method are compared to obtain co-occurrence matrices. The area of surface of change of European WT fleet is identified thanks to the analysis of Copernicus Sentinel-2 satellite images from 2015 to 2023. Correlation between this area and WT capacity, or hub height, or rotor diameter will be evaluated, to have a parametric LCI, thanks to a surface change file. The change due to road construction is based on legal minimal distance to road for construction of new WT in European countries. For occupation, the lifetime of the WT is used, and its influence will be assessed in a sensitivity analysis. The new model will be open-source and also integrated in an online interactive map that optimizes the position and characteristics of WT and shows the diverse impacts of WT, including an LCA model as well as impacts on noise, landscape, among others. The co-occurrence matrices match, in some ecoregions, still need to be studied in detail because the different resolutions and time of data collection of the datasets, as well as specific patterns of ecoregions may be the source of differences. The surface area change was analyzed for more than 78,000 WT in Europe. Depending on the distance buffers, concave hulls and convex hulls assessed around the WT, between 22m²/WT and 152m²/WT are changed after WT construction. This model will be used to evaluate for the first time the European WT fleet impact on biodiversity in terms of potential disappeared fraction (PDF) of species per unit of energy produced.

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5.08.P-We438 Calculation of Characterization Factors for Life Cycle Impact Assessment of Biocides Using USEtox

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Biocides are chemicals used to control detrimental organisms for products. While beneficial, they pose risks to human health and ecological balance. Risk assessment is necessary and legally required before market entry. Incorporating a life cycle perspective in their analysis offers a key advantage: it allows

comparing benefits of extending a product's lifespan during storage and use with the environmental impacts of resource consumption, manufacturing, and spoilage. Life Cycle Assessment (LCA) is a widely used methodology for such analysis. However, LCA databases lack sufficient data on chemicals due to the vast number of substances, making it difficult for companies and researchers to fully leverage LCA. Specifically, quantifying biocides impacts on human toxicity and ecotoxicity is constrained by this data gap. This research investigates whether characterization factors for biocides can be calculated using readily available data from existing research, development processes, or other sources.

The calculation of characterization factors (CFs) relies on three components as defined by the USEtox methodology: Fate Factor, Exposure Factor and Effect Factor. The required data include physico-chemical properties, propagation routes, and hazardousness. Previous research suggests that the scarcity of CFs for chemicals is largely due to a lack of experimental data necessary for their computation. This study proposes to explore readily accessible sources, such as data used in risk assessments, enabling companies to calculate CFs and perform LCA.

The study focuses on calculating CFs for PT 6 biocides (preservation during storage). The active substance used as a case study is a mixture of C(M)IT and MIT. The primary source of data for the calculation of effect and exposure factors is the Authority Report for the review program of existing active substances (Directive 98/8/EC). The fate factor is derived using the USEPA EPI Suite tool. As validation process, the methodology will be tested on commonly known VOCs such as formaldehyde, benzene, and ethylene glycol to identify potential errors and uncertainties.

This study aims to determine whether private entities can independently calculate CFs. Given the diversity of chemical substances, it is impractical to centralize their CF development. Allowing chemical producers to calculate CFs independently can empower them to assess the environmental impact of their products efficiently and support sustainable market entry.

5.08.P-We439 Environmental Impact Assessment of Nanotechnology Using Life Cycle Assessment (LCA)

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Nanomaterials, as a key area of nanotechnology, have gained significant attention for their unique functionalities, driving advancements in diverse scientific and industrial fields such as medicine, semiconductors, and energy. Simultaneously, the potential human and ecological toxicity of nanomaterials, arising from their size and surface properties, has led to the development of risk-based safety management policies and related technologies. Recently, beyond safety considerations, there has been a growing focus on assessing the environmental impacts of nanomaterials across various categories, including resource and energy consumption, carbon and greenhouse gas emissions, and the release of environmentally hazardous substances. These assessments aim to evaluate and ensure the sustainability of materials and technologies. The sustainability of nanomaterials appears to be properly assessed using the life cycle assessment (LCA) framework, which evaluates impacts across the entire life cycle, from production to disposal. This study reviewed research cases to establish the application of LCA for nanomaterials. Key data sources, inventory utilization, assessment categories, and major findings in LCA studies on nanomaterials were systematically examined. To integrate nanotechnology within the LCA framework effectively, three primary research areas need to be addressed. First, it is essential to adopt appropriate functional units that account for all additional functionalities of manufactured nanomaterials (MNMs) compared to their bulk counterparts. Second, the development of life cycle inventory (LCI) data for the production of MNMs is crucial, along with methodologies to qualitatively and quantitatively model the eventual release of MNMs throughout their lifecycle. Third, characterization factors for released MNMs must be developed, as they are critical for incorporating MNM emissions into life cycle impact assessment (LCIA). The proposals outlined in this study will contribute to establishing a framework for assessing the safety and sustainability of nanomaterials, advancing their responsible development and application.

5.08.P-We440 New Advances to Assess Biodegradation and Toxicity of Alternative Environmentally Friendly Polymers

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The inherent toxicity linked to plastic ubiquitous presence in the environment has received a growing interest in the last decades. Even though laws, policies and scientific projects addressing plastic pollution and its impact have recently flourished, very few concerned plastic risk assessment (i.e. linking plastic exposition and their effects on ecosystems) taking into account conventional or biobased, recycled or biodegradable polymers. Our objective was to develop new methods to assess plastic end-of-life on marine environment based on biodegradability assays and toxicity tests on marine organisms in order to develop environmental impact indexes relevant for Life Cycle Analysis.

On the basis of field and laboratory experiments, we tested different conditions to follow the biodegradation of polymers by natural marine bacterial communities (named *plastisphere*). We tested several media and conditions to measure the biodegradability of biosourced and petroleum based polymers comparatively to cellulose standard, in real marine and aquatic conditions. On the other hand, we developed methods to measure the impacts of plastics at different trophic levels of the marine environment, based on ecotoxicological studies on communities in their natural environment at the molecular, cellular, organ, individual and population levels. The methodological limits of toxicity tests, generating a partial assessment of the impact of microplastics on marine organisms were tested including several limits such as concentration, size, shape, chemical composition of plastics. Finally, relevant standards were tested to evaluate plastic toxicity to assess the effective risk of alternative and conventional plastics in marine biodiversity.

5.08.P-We441 Evaluation of the USEtox Method for Use in the Sustainability Assessment of Selected Packaging

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The European Commission has developed the Product Environmental Footprint (PEF) to assess the impacts of a product using a standardised and harmonised LCA-based approach. The concept contains specific rules for the assessment of impacts in 16 categories based on existing methods. For the toxicity-related categories - human cancer toxicity, human non-cancer toxicity and freshwater ecotoxicity - the PEF refers to the science-based consensus model USEtox. This model calculates the effects of chemicals individually based on fate, exposure, and the impact on humans or ecosystems. The European Commission subjects all methods used in the PEF to a robustness assessment that includes a completeness check, a sensitivity analysis and a consistency check. Based on this robustness assessment, this work tests the applicability and robustness of the USEtox method for assessing the toxicity of the packaging materials used in a yoghurt cup to evaluate whether the method is suitable for the toxicity assessment of packaging materials, particularly food contact materials. For the completeness check, the latest USEtox version (v2.13) is analysed, and the method's approach and results are compared with different approaches of toxicity assessments. In the sensitivity analysis, the results of different USEtox versions are calculated to assess the dependence of the results on the differences in the methods. In the third step, a consistency check is carried out by comparing the results with various ecoinvent versions to analyse the consistency of the data. The results of the assessment show that USEtox differs from other approaches in some aspects, mainly attributable to the hazard-based concept. Although this approach is scientifically based, it is only suitable for assessing the toxicity of packaging such as the yoghurt cup to a limited extent, as it does not consider the utilisation phase or actual exposure. In addition, the results vary depending on the USEtox version and database used, which makes it difficult to draw reliable conclusions. Errors in the database affecting certain chemical streams have also recently been uncovered, suggesting that a careful review of the data used is also needed. Therefore, further research is needed to resolve these uncertainties.

5.08.P-We442 Refining Ecotoxicity Assessment in Life Cycle Assessment: Bridging Data Gaps for Comprehensive Environmental Impact

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Chemical substances released into the environment are distributed across the various environmental compartments based on their physicochemical properties and interactions. The presence of these substances has been shown to cause ecotoxicological impacts in different environmental compartments, with the extent of these impacts depending on factors such as substance fate, exposure -environmental concentrations, residence time, the mode of action and the sensitivity of the species and communities- and environmental effect on species.

A product's life cycle begins with raw material extraction and energy generation, followed by production, packaging, distribution, use, maintenance, and ultimately recycling, reuse, recovery, or disposal occurring

chemical emissions to soil and water in each stage.

Ecotoxicity is one of the impact categories covered in the life cycle assessment (LCA) methodology. It involves translating the total environmental emissions of chemicals associated with the production, use, and end-of-life of a good or service into a measure of their potential ecotoxicological impact. However, the current available life cycle impact assessment methods address only a limited amount of chemicals, leaving a significant proportion of chemicals to which ecosystems may be exposed unassessed.

This study presents a systematic review to understand how ecotoxicity has been addressed within the LCA. It explores the development of environmental impact mechanisms on water and soil, identifies the substances and organisms considered, and identifies the species and extrapolation procedures employed in the absence of data. Furthermore, the study proposes future directions for enhancing LCA and ecotoxicity assessment practices.

The limited availability of toxicity data for environmental chemicals highlights the importance of this study. By providing a comprehensive analysis of the current limitations of LCA and ecotoxicity impact indicators, this study highlights the need to increase the life cycle inventory datasets of substances' ecotoxicity and improve the interlink between ecotoxicity concentrations and organism resilience.

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5.09 LCA for Decision-Making, Communication and Reporting

5.09.T-01 Using Absolute Environmental Sustainability Assessment and Mid-To-Endpoint Modelling to Identify the Most Relevant Impact Categories to Include in a Building LCA

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The construction sector in Europe is increasingly documenting its impact on climate change through life-cycle assessment (LCA). The movement is bolstered by legislation mandating the calculation of greenhouse gas emissions from new buildings. While there has been a strong emphasis on assessing climate change impacts, other environmental impacts have often been neglected. This study identifies the most relevant midpoint impact categories to include in LCAs on buildings through two novel complementary approaches. The two approaches assess relevance based on the severity of impacts: absolute environmental sustainability assessment (AESA) and mid-to-endpoint contribution analysis. The approaches are applied to 40 residential buildings covering 370,000 m². For the AESA approach, impact categories for which case buildings exceeded their allocated carrying capacity were considered relevant, while the impact categories contributing most to endpoint damages were considered relevant for the mid-to-endpoint approach. The study's characterization factors and allocation principles were tested in a robustness analysis. Climate change, land use, and fine particulate matter were relevant according to both approaches and showed a high robustness towards changes in the set of characterization factors and allocation principles. Freshwater ecotoxicity, freshwater eutrophication, human carcinogenic toxicity, and mineral use were less robust considering the choice of life-cycle impact methods and allocation principles. Our approach aims to help the building industry find a middle ground between only reporting LCA results for climate change, which could lead to burden-shifting, and systematically reporting LCA results for all midpoint impact categories, which could lead to decision paralysis.

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5.09.T-02 Allocation in ISO, GHG Protocol, and Environmental Footprints

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The flora of ISO standards related to life cycle assessment (LCA) is growing organically. Meanwhile, the

Greenhouse Gas Protocol has become a de facto standard for many industrial companies, and the EU has established Environmental Footprint as the framework to use in Europe. These documents all refer to the allocation hierarchy in ISO 14044, but present a variety of interpretations and even deviations from this hierarchy. A line of conflict concerning the interpretation of system expansion is apparent; however, the standards also diverge in the interpretation of allocation that reflects underlying physical relationships or other relationships.

We present the points of conflict between the standards. We also present ambiguous points in the documents. We discuss the accuracy of different interpretations of the allocation hierarchy as well as their relevance to decision-making. Finally, we discuss changes that can be made in the allocation hierarchy to make LCA a more effective tool in decision-making.

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5.09.T-03 Module D in Environmental Product Declarations (EPDs): Sorting the Weed of Misinterpretation

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Communicating and reporting Module D in Environmental Product Declarations (EPDs) is recognized as challenging, especially for individuals unfamiliar with Life Cycle Assessment (LCA). Module D, as defined in the EN 15804+A2 standard, involves hypothetical environmental credits rather than actual life cycle impacts. As a result, misleading impressions of environmental benefits may unintentionally be created. For example, negative values from Module D can make a product appear more environmentally friendly than it actually is when compared to the concrete stages A1 to C, which cover processes from raw material extraction to disposal. Challenges associated with Module D modeling are introduced by differences in recycling practices, regional variations, and inconsistent data quality. Additionally, reliance on general assumptions rather than location-specific data complicates the alignment of LCA objectives with practical applications. Since Module D is not an actual life cycle stage but instead accounts for credits from recycled or reused materials in other life cycles, confusion among non-LCA stakeholders is often increased.

When EPDs for products such as steel pipes, hot-rolled steel bars, and ready-mix concrete were analyzed, greater variability is observed in Module D values compared to those of Modules A1-A3. The importance of a standardized LCA approach is therefore highlighted to ensure that results are consistent and comparable. Practical aspects of applying Module D in EPDs include defining end-of-life waste scenarios, assessing material quality differences, and recycling. This paper aims to provide guidance on simplifying Module D modeling, with an emphasis on standardized LCA practices to effectively address data and communication challenges. By ensuring transparency and minimizing the risk of greenwashing, more reliable sustainability information for decision-making and policy compliance can be supported.

5.09.T-04 Decision-Oriented Choice for Electricity Supply Modelling in Attributional LCA: Location-Based or Market-Based Approach?

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Most products consume electricity, either for their production or their use phase. Therefore, for assessing their life cycle impacts through an attributional LCA, accurately modelling their electricity supply is important. Two main accounting approaches are used in LCAs: (1) the location-based approach, considering territorial electricity mixes (either at the national or at the regional level) and (2) the market-based approach considering market instruments such as Energy Attribute Certificates (EACs, e.g. Guarantees of Origin (GO) in Europe) complemented by residual mixes (as provided for instance by the Association of Issuing Bodies (AIB) in Europe).

As an introduction, basic physics of electricity production and distribution will be presented. Then, both the location-based and the market-based approaches will be presented, and their main pros and cons discussed. Based on this analysis, we will propose guidelines to select an approach that better matches the objectives of most Industry attributional LCAs, which are: product eco-design, product comparison and publication of environmental declarations.

In most cases, the use of the location-based approach avoids accusations of greenwashing and ensures a consistent use of datasets in the foreground and background of an LCA, whereas the use of the market-based approach can face accusations of resource shuffling and inconsistencies within one LCA (e.g. geographical and temporal inconsistencies between electricity production and consumption and double counting of the energy supply processes covered by EACs).

As a conclusion, we can say that different accounting approaches serve different purposes and before

choosing an accounting method, one should be clear about what is the goal and scope of its LCA. For product eco-design and comparison, the location-based approach should be favored, because it provides a better understanding of the product value chain processes and their related environmental impacts and the potential impact transfers for different product design options. However, the market-based approach can be used for communication purposes, provided the EAC systems evolve to encourage better matching of the physical reality of electricity production and consumption. Meanwhile, it is of utmost importance that the electricity supply modelling hypotheses and implementation are transparent to the recipients of the LCA reports, to allow for better understanding and comparability.

5.09.P-We443 From Opportunities of Industrial LCA Usage to Data Specification Challenges: The Example of Winter Sport Hard Goods

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The motivation, chances and challenges of using Life Cycle Assessment (LCA) in research and industry are different with a lack of studies emphasizing on specificities of industrial usage. Within multiple challenges of using LCA in industry, data collection internally and from external suppliers proves a major obstacle. Data specifications are a necessity within methodological frameworks and a key aspect in decision-making fostering a company's journey towards more sustainable production.

This study aims to elaborate on the motivation, status quo and future application of LCA in the winter sport industry in Austria, which has been selected as an example which is representative for many small and medium enterprises (SME) in the European legal context. Additionally, options and limitations to collect, consider and use specific data from suppliers in product LCA are discussed using two different background databases.

Bilateral interviews have been conducted with five companies comprising open questions to i) determine the general situation and attitude towards sustainability, and ii) the status quo of LCA in the companies, as well as iii) a SWOT analysis. Concerning data specification, a collection template has been developed, filled and discussed with key suppliers for an exemplary intermediary product; an injection-molded part made from polypropylene. The environmental impacts are calculated for a default and a specified scenario using Environmental Footprint (EF3.1) and ecoinvent 3.10 cut-off databases.

The interviewed companies shared similar motivations and usage of LCA results, which is currently mostly applied for internal purposes, such as deepening the understanding of production processes and product development, and only occasionally used for sustainability reporting and to communicate with external stakeholders. The shared challenges are lack of data availability, limited comprehensiveness of standard, and uncertainty on requirements and formats for external communication, including (future) legal development.

The data specification exercise demonstrated that not only data collection but especially data analysis and implementation in the LCA model require additional effort with differences in feasibility between the databases. On the other hand, the gain in accuracy provides a more differentiated picture for the 16 impact categories of the EF3.1 impact assessment method and promotes communication and collaboration along the value chain.

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5.09.P-We444 Life Cycle Costing Aligned with Life Cycle Assessment as part of Life Cycle Sustainability Assessment: Flexibility and Challenges Depending on the Decision-Making Context

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Existing life cycle sustainability assessments (LCSAs) suffer from a lack of comprehensiveness, consistency and practical tools for implementation. A robust and operational methodology for LCSA of products was developed in the EU-funded ORIENTING project (www.orienting.eu; 2020-2024) in which life cycle costing (LCC) covers the economic dimension. An LCC methodology was developed that (a) suits LCSA purposes not tailored to a specific type of product, (b) is aligned with the environmental Life Cycle Assessment (LCA) part of LCSA, (c) explicitly addresses double counting notably when using the same data as in the Life Cycle Inventory (LCI), (d) is applicable to different stakeholder needs and (e) addresses externalities. While it was designed for LCSA, it can also be used stand-alone.

To achieve consistent decision-making, an environmental LCC approach is adopted, i.e. an LCC aligned with LCA. This includes distinguishing the same set of life cycle stages and the consideration of double counting by taking either the so-called own realities position or adjusting LCA (e.g. disregarding abiotic resource uses or distinguishing emissions with and without costs).

Decision context-specific flexibility is implemented in terms of considering discounting or positive cash flows and thus the choice of the economic indicator. This also depends on the defined goal and scope, and thus the chosen stakeholder perspective. Three stakeholder perspectives are distinguished: the producer, the consumer and society.

The decision context can also influence data collection strategies, as exemplified by how different stakeholders would fill the proposed cost breakdown structure.

The LCC methodology was applied in two case studies, one on the business-to-business (B2B) product beverage carton package and one on the consumer product outdoor wool jacket. Amongst other things, the case studies demonstrate how data confidentiality can impact the way in which results can be presented. In the jacket case, the results of a contribution analysis could be made public. In the beverage carton package case, results based on primary data could not be shared for confidentiality reasons (especially prevailing for B2B products), while results based on publicly available data were misleading and thus not shared. This highlights that while LCC is well suited for internal decision making, public communications might be hampered.

The study has already been published: <https://rdcu.be/dOKRe>.

5.09.P-We445 Knowledge Graphs for LCA: Streamlining Consistent Reporting

Didier Beloin-Saint-Pierre¹, Alexander Kirsten¹, Joana Francisco Morgado¹, Daniel Lachat¹, Jan Grau², Kimberly Garcia² and Roland Hischer¹, (1)EMPA - Swiss Federal Laboratories for Materials Science and Technology, Switzerland, (2)University of St. Gallen, Institute for Computer Science, Switzerland

Recent developments of the European regulation (e.g. corporate sustainability reporting directive) are creating pressure to increase the capacity to make more life cycle assessment (LCA) studies in the near future even if there is now a shortage of experts and consultants to do this work. We therefore need to find faster ways to carry out and review LCA studies for organisations and products.

Meanwhile, many associations and institutes have proposed various standards for environmental assessments (e.g. product environmental footprint) or frameworks to exchange data (e.g. pathfinder initiative). The differences between these standards and frameworks hinder simple exchange of consistent data between companies along value chains.

In this context, it is interesting to see that most of the environmental assessment standards are inspired by the foundational work presented in the ISO 14040-14044 documents. This common basis can help in identifying common rules that have been used by experts and consultants to check if different data sources and LCA studies can be consistently compared or combined to create models of various value chains.

We thus propose to build knowledge graphs (KGs) for LCA standards and related databases utilising the common key concepts defined in the ISO 14040-14044 documents. The KGs provide a machine readable and understandable description of rules that need to be followed to ensure that LCA studies are carried out consistently even for different guidelines. These KGs can then be used to automatically make consistency checks and translate LCA studies in the format of different standards (e.g. respecting specific disclosure requirements).

Two connected KGs are created to enable the consistency checks and translation of LCA studies. The first KG lists the rules of LCA standards that need to be checked so that assessed environmental impacts of organisations, products or services are valid for a selected assessment context. A second KG informs on the content and formats of most LCA data sources (e.g. studies and databases). The two KGs are then connected to enable the evaluation of the consistency between different studies that might be considered comparable. Moreover, the KGs allow translating the results of such studies to different formats.

The developed KGs thus address key challenges that currently hinder exchanges of consistent LCA data between stakeholders of supply chains, which will hopefully lead to more consistent reporting.

Disclaimer/Disclosure: Acknowledgement - The authors thank Innosuisse for funding their research in the context of the WISER flagship (PFFS-21-72, Reference number: 2150009487).

5.09.P LCA for Decision-Making, Communication and Reporting

5.09.P-We443 From Opportunities of Industrial LCA Usage to Data Specification Challenges: The Example of Winter Sport Hard Goods

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The motivation, chances and challenges of using Life Cycle Assessment (LCA) in research and industry are different with a lack of studies emphasizing on specificities of industrial usage. Within multiple challenges of using LCA in industry, data collection internally and from external suppliers proves a major obstacle. Data specifications are a necessity within methodological frameworks and a key aspect in decision-making fostering a company's journey towards more sustainable production.

This study aims to elaborate on the motivation, status quo and future application of LCA in the winter sport industry in Austria, which has been selected as an example which is representative for many small and medium enterprises (SME) in the European legal context. Additionally, options and limitations to collect, consider and use specific data from suppliers in product LCA are discussed using two different background databases.

Bilateral interviews have been conducted with five companies comprising open questions to i) determine the general situation and attitude towards sustainability, and ii) the status quo of LCA in the companies, as well as iii) a SWOT analysis. Concerning data specification, a collection template has been developed, filled and discussed with key suppliers for an exemplary intermediary product; an injection-molded part made from polypropylene. The environmental impacts are calculated for a default and a specified scenario using Environmental Footprint (EF3.1) and ecoinvent 3.10 cut-off databases.

The interviewed companies shared similar motivations and usage of LCA results, which is currently mostly applied for internal purposes, such as deepening the understanding of production processes and product development, and only occasionally used for sustainability reporting and to communicate with external stakeholders. The shared challenges are lack of data availability, limited comprehensiveness of standard, and uncertainty on requirements and formats for external communication, including (future) legal development.

The data specification exercise demonstrated that not only data collection but especially data analysis and implementation in the LCA model require additional effort with differences in feasibility between the databases. On the other hand, the gain in accuracy provides a more differentiated picture for the 16 impact categories of the EF3.1 impact assessment method and promotes communication and collaboration along the value chain.

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5.09.P-We444 Life Cycle Costing Aligned with Life Cycle Assessment as part of Life Cycle Sustainability Assessment: Flexibility and Challenges Depending on the Decision-Making Context

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Existing life cycle sustainability assessments (LCSAs) suffer from a lack of comprehensiveness, consistency and practical tools for implementation. A robust and operational methodology for LCSA of products was developed in the EU-funded ORIENTING project (www.orienting.eu; 2020-2024) in which life cycle costing (LCC) covers the economic dimension. An LCC methodology was developed that (a) suits LCSA purposes not tailored to a specific type of product, (b) is aligned with the environmental Life Cycle Assessment (LCA) part of LCSA, (c) explicitly addresses double counting notably when using the same data as in the Life Cycle Inventory (LCI), (d) is applicable to different stakeholder needs and (e) addresses externalities. While it was designed for LCSA, it can also be used stand-alone.

To achieve consistent decision-making, an environmental LCC approach is adopted, i.e. an LCC aligned with LCA. This includes distinguishing the same set of life cycle stages and the consideration of double counting by taking either the so-called own realities position or adjusting LCA (e.g. disregarding abiotic resource uses or distinguishing emissions with and without costs).

Decision context-specific flexibility is implemented in terms of considering discounting or positive cash flows and thus the choice of the economic indicator. This also depends on the defined goal and scope, and thus the chosen stakeholder perspective. Three stakeholder perspectives are distinguished: the producer, the consumer and society.

The decision context can also influence data collection strategies, as exemplified by how different

stakeholders would fill the proposed cost breakdown structure.

The LCC methodology was applied in two case studies, one on the business-to-business (B2B) product beverage carton package and one on the consumer product outdoor wool jacket. Amongst other things, the case studies demonstrate how data confidentiality can impact the way in which results can be presented. In the jacket case, the results of a contribution analysis could be made public. In the beverage carton package case, results based on primary data could not be shared for confidentiality reasons (especially prevailing for B2B products), while results based on publicly available data were misleading and thus not shared. This highlights that while LCC is well suited for internal decision making, public communications might be hampered.

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5.09.P-We445 Knowledge Graphs for LCA: Streamlining Consistent Reporting

Didier Beloin-Saint-Pierre¹, Alexander Kirsten¹, Joana Francisco Morgado¹, Daniel Lachat¹, Jan Grau², Kimberly Garcia² and Roland Hischier¹, (1)EMPA - Swiss Federal Laboratories for Materials Science and Technology, Switzerland, (2)University of St. Gallen, Institute for Computer Science, Switzerland

Recent developments of the European regulation (e.g. corporate sustainability reporting directive) are creating pressure to increase the capacity to make more life cycle assessment (LCA) studies in the near future even if there is now a shortage of experts and consultants to do this work. We therefore need to find faster ways to carry out and review LCA studies for organisations and products.

Meanwhile, many associations and institutes have proposed various standards for environmental assessments (e.g. product environmental footprint) or frameworks to exchange data (e.g. pathfinder initiative). The differences between these standards and frameworks hinder simple exchange of consistent data between companies along value chains.

In this context, it is interesting to see that most of the environmental assessment standards are inspired by the foundational work presented in the ISO 14040-14044 documents. This common basis can help in identifying common rules that have been used by experts and consultants to check if different data sources and LCA studies can be consistently compared or combined to create models of various value chains. We thus propose to build knowledge graphs (KGs) for LCA standards and related databases utilising the common key concepts defined in the ISO 14040-14044 documents. The KGs provide a machine readable and understandable description of rules that need to be followed to ensure that LCA studies are carried out consistently even for different guidelines. These KGs can then be used to automatically make consistency checks and translate LCA studies in the format of different standards (e.g. respecting specific disclosure requirements).

Two connected KGs are created to enable the consistency checks and translation of LCA studies. The first KG lists the rules of LCA standards that need to be checked so that assessed environmental impacts of organisations, products or services are valid for a selected assessment context. A second KG informs on the content and formats of most LCA data sources (e.g. studies and databases). The two KGs are then connected to enable the evaluation of the consistency between different studies that might be considered comparable. Moreover, the KGs allow translating the results of such studies to different formats. The developed KGs thus address key challenges that currently hinder exchanges of consistent LCA data between stakeholders of supply chains, which will hopefully lead to more consistent reporting.

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5.09.P-We446 Recent Advances and Future Prospects of Optimization-Based Decision Support in LCA using PULPO

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Life Cycle Assessment (LCA) has become a cornerstone of environmental decision-making. However, the complexity of contemporary sustainability challenges raises the need to go beyond simple static production system assessments. To address this, advanced tools such as dynamic and scenario assessments have been developed to extend LCA's capabilities, but gaps remain particularly in optimization-based decision support.

To bridge this gap, we developed PULPO, a method and tool that effectively adds Life Cycle Optimization (LCO) to the LCA toolbox, enabling practitioners to identify optimal production system maximizing performance in some impact categories. This approach eliminates the need for exhaustive scenario screening by streamlining the process of finding the best configurations, enabling the automatic exploration of vast numbers of scenarios in short CPU times. Beyond this, PULPO supports advanced considerations such as system constraints, including capacity limits, material availability, and regulatory

or environmental flow restrictions, which traditional LCA approaches often struggle to incorporate. Additionally, PULPO facilitates the simultaneous variation of both foreground and background processes, capturing essential feedback loops crucial for emerging technologies and large-scale decision-making. In this contribution, we present the latest advancements in PULPO, detailing updates that enhance user experience and computational features. Improvements include a simple graphical user interface for basic optimization tasks and the incorporation of integer decision-making capabilities. Through the latter, users can now specify minimum capacities for production system elements, exclude specific configurations, and generate ranked lists of candidate solutions. These enhancements make PULPO more accessible and powerful, providing decision-makers with the tools to answer previously unsolvable questions.

We believe that PULPO's open-source framework empowers the LCA community to address complex decision-making challenges and encourages collaborative development tailored to emerging needs. This tool not only advances the practical use of Life Cycle Inventory (LCI) data but reinforces its potential to shape impactful policy and strategic decisions in environmental management.

5.09.P-We447 Methodological Diversity in Carbon Footprint Assessment: Challenge for Decisionmakers in the Packaging Sector

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The Corporate Sustainability Reporting Directive (CSRD) requires companies to document environmental parameters, including greenhouse gas emissions. How greenhouse gases must be accounted for is specified in more detail in the European Sustainability Reporting Standards (ESRS). There it is stated that the accounting should be compliant with the Greenhouse-Gas-protocol and emission factors from the Intergovernmental Panel on Climate Change (IPCC) should be used. In addition, the European commission's recommendation on Product Environmental Footprint (PEF) or EN ISO 14064-1:2018 standard is mentioned as the framework under which a greenhouse gas assessment shall take place. These instruments differ in scope and content, offering flexibility in their application.

For end-of-life allocation of burdens and credits for example, methods like the Circular Footprint Formula (described in PEF) or the cut-off approach can be used. LCIA methods for assessing greenhouse gases, such as EF3.1 or ReCiPe Midpoint (H), may yield different results despite both being IPCC-based. Variations in system boundaries, databases, transport distances, or end-of-life rates can significantly impact results. Therefore, it is hypothesised that even with ESRS conformity, calculations of greenhouse gases for packaging materials in different studies might lead to varied conclusions due to differing parameter settings and framework conditions.

To explore this hypothesis, life cycle analyses and carbon footprint calculations of packaging are compared in terms of coherence and the different methodological approaches are contrasted. Packaging materials (plastic, aluminium, paper, steel and glass) are then analysed regarding different parameters to show the level of differences (parameters: end-of-life allocation, LCIA-method, system boundaries).

By adjusting the parameters described, major changes in the results occur. This means that the use of different but recognised methods gives the impression of direct comparability, although the results taken out of context can result in misleading or incorrect conclusions. This poses challenges for organisations and decision makers who need to act based on the results. To create comparability and achieve the objective of the CSRD, it would be necessary to standardise the methods used to calculate the carbon footprint. Therefore, this study compares the different standards, identifies potentials, and proposes improvements in standardisation.

5.09.P-We448 A Novel Multidimensional Impact Assessment Framework to Bridge the Gap Towards a Truly Sustainable and Circular Bio-Based Industry

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The transition towards a bio-based economy will deliver significant environmental and economic benefits. However, bio-based production systems need to address several challenges related to biological feedstocks (e.g., seasonality, variability on physicochemical properties, or spatial distribution). In addition, there is a need for assessment methods or schemes that integrate environmental, socioeconomic and circular issues to ensure a truly sustainable performance of biological feedstocks.

In the framework of the Horizon Europe BIORECER project (101060684), it was developed a multidimensional impact framework addressing environmental performance and circularity of biological resources based on a Life Cycle Assessment (LCA) approach. The proposed framework provides guidelines on data requirements to holistically evaluate the sustainability of bio-based supply chains. Thus, existing impact assessment methodologies, sustainability standards applicable to biological

resources, and circularity indicators were reviewed to identify those requirements (up to 52) and criteria that cover both sustainability and circularity.

As a result, an integrated set of indicators intended to be used in bio-based production systems-- to practically assess the sustainability along a bio-based supply chain was established. In addition, the proposed framework will be the basis for the adaptation of current certification schemes since it includes novel criteria for certifying the sustainability of biological resources. The proposed framework has been tested and validated in four case studies covering different biological feedstocks (i.e., sewage sludge, seafood waste, organic fraction of municipal solid waste, agro-waste, and forest industry sub-products), providing promising results.

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5.09.P-We449 Beyond Zero-Risk Assumption: Integrating Accident Impacts into LCA of Hydrogen Delivery Options

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As the European Union prepares to import large quantities of low-carbon hydrogen, the optimal form of delivery remains uncertain. A previous life cycle assessment (LCA) by the Joint Research Centre of the European Commission compared the environmental sustainability of various delivery options, including gaseous hydrogen, liquid hydrogen, and chemical carriers such as ammonia, methanol, synthetic natural gas, and liquid organic hydrogen carriers. Although liquid hydrogen emerged as the most environmentally sustainable option for European territories, the study assumed a zero-risk scenario, neglecting the potential environmental impacts of accidents during delivery. This research addresses this limitation by estimating accident risks and incorporating their environmental consequences into the LCA. It is highlighted that excluding accidents from the environmental profile of hydrogen carriers provides an incomplete picture of their sustainability. We argue that a more systematic approach to integrating risk assessments into comparative LCAs is essential for informed decision-making in the transition to a low-carbon hydrogen economy.

5.09.P-We450 Variability of Organizational Carbon Footprint Depending on Accounting Standards and Data Sources: A Case Study

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Accurate organizational carbon footprinting is necessary to efficiently plan carbon neutrality of corporations. But different methodological choices are possible to conduct the calculations, in terms of accounting standards companies mostly use the GHG Protocol or the organizational LCA standard ISO/TS 14072 and emission factor sources - derived either from LCA, environmentally-extended input-output (EEIO) analysis, or a combination of both. The impact of the chosen accounting method and data on the estimated carbon footprint and its reliability, however, remains poorly understood. This study aims to illustrate these methodological choices influence estimated organizational carbon footprints on a case study.

We conduct 4 organizational carbon footprint assessments. We rely either on the ISO/TS 14072 standard or the GHG Protocol. We use different emission factors from ecoinvent (EI) v3.8, from OpenIO-Canada v2.0, or from the WRI resources provided online. The activity data used, provided by the company, include both economic and physical data. The physical data encompasses energy-related information and mass-based data (materials purchased). The economic data correspond to the physical inventories.

Results show that the organizational carbon footprint calculated vary by a factor of 4.6: 2350 tCO₂e for LCA combined to OpenIO-Canada, 5230 tCO₂e for the GHG Protocol with the WRI data, 10000 tCO₂e for the LCA combined to ecoinvent, and 10700 for the GHG Protocol combined to ecoinvent. Hotspots also vary depending on the accounting method, the most important contributor either being business travel (42%) for the LCA - OpenIO-Canada method, use of products (36%) for the GHG Protocol WRI method, and stainless steel purchases when using EI data.

Based on this case study, estimated organizational carbon footprints are highly dependent to accounting methodological choices especially the emission factors used. The use of EEIO emission factors for potential hotspot contributors is not recommended due to the high aggregation of the commodities represented in these databases. Nevertheless, EEIO data can usefully complete the assessment scope when

process-based data are not available, activity data are only provided in economic units, or resources to produce the accounting are very limited.

5.09.P-We451 Comparative Assessment of Environmental Performance: Green Roofs vs Conventional Roofs

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The growth of urban infrastructures and cities without sustainable planning has heightened vulnerability to extreme weather events, such as flooding and urban heat island effects. The building sector accounts for 37 % of global greenhouse gas emissions, and requires high energy consumption for heating and cooling, it has an important potential to implement sustainable strategies. The European Union has encouraged the synergy between green and gray infrastructures, emphasizing green roofs (GRs) as a key strategy to enhance urban resilience and improve buildings thermal performance. GRs, vegetated structures above buildings, can be classified into extensive and intensive types, based on their weight, type of vegetation, and maintenance needs. The Life Cycle Assessment (LCA) methodology has been applied to evaluate GRs environmental impacts. However, results diverge due to a lack of standardized technical guidelines. This study quantifies the environmental impacts of extensive and intensive GRs, designed according to expert-recommended technical guidelines, in comparison with a conventional flat roof (CR) in Portugal, applying the LCA methodology. The study also identifies the life cycle stage that most contributes to the roofs' total environmental impacts. Nine scenarios were developed: four extensive GRs, four intensive GRs, and one CR, varying the type and quantity of materials in the GRs substrate layer. The functional unit is 1 m² of the roof with a lifespan of 40 years. All scenarios were assessed based on a cradle-to-grave approach including materials, construction, use and End-of-Life (EoL) stages. Overall, comparing GR with CR, CR presents the best environmental performance for all impact categories analysed, due to materials used in GR substrate. Comparing GR types, extensive GR has lower environmental impacts than intensive GRs, with reductions ranging from 34 % in Ozone formation - human health to 59 % in marine eutrophication, mainly attributed to the highest material needed for intensive GR. The hotspot analyses showed that the materials stage was the main contributor to the environmental impacts for the nine scenarios, except for the freshwater eutrophication and marine eutrophication impact categories, in which the EoL is the hotspot for the GR, due to the EoL of the substrate materials to a landfill. Future research should further explore the potential benefits of GRs, particularly how GRs affect the thermal performance of buildings.

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5.09.P-We452 Life Cycle Assessment of Reusable Bio-based Cups as a Substitute for Single-use Plastic at Festivals

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Single-use plastic materials present a significant threat to the environment due to their contribution to resource depletion and plastic pollution. Fossil-based plastic products, which dominate the market, take an exceedingly long time to decompose [1]. The tourism industry stands out as a major contributor to the proliferation of single-use plastics, most notably typified by touristic sites experiencing a surge in plastic waste during peak seasons. Addressing this issue requires innovative and sustainable solutions that not only mitigate plastic waste but also promote a circular economy and zero-waste alternatives. The implementation of such measures is crucial for achieving sustainability, which encompasses environmental, economic, and social dimensions. To evaluate the effectiveness of these solutions, it is essential to quantify their impacts across all three pillars with holistic evaluation methods. The solution proposed in this study are reusable bio-based cups for beer consumption at festivals and other large-scale events, serving as a substitute for conventional single-use polypropylene cups. The analysis focuses on evaluating the environmental, economic, and social impacts of implementing this alternative on a large scale in comparison to a business-as-usual approach, using a case study of a widely visited music festival event site.

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5.09.P-We453 The EcoBeautyScore Association's Approach to Quantifying Data Representativeness of Cosmetic Product Formulas and Its Applications in Environmental Scoring Calibration and Future Data Development

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The EcoBeautyScore (EBS) Association*, comprising over 70 global cosmetic companies and associations, has developed a unified environmental footprint measurement and scoring system for cosmetic products over the past three years. This system is based on Life Cycle Assessment (LCA), recognized by the European Commission (EC) as the most effective method for assessing and comparing the environmental footprint of products and services. The LCA methodology aligns with the Product Environmental Footprint (PEF) guideline initiated by the EC in 2013. Moreover, the method was enhanced to suit the cosmetic specificities.

To ensure meaningful comparisons, products being compared must fulfil the same function. As such, we segmented products across over 30 basic services, such as hair washing and face moisturizing and treatment. In each segment, high product diversity dictated the need for a statistical approach to: (1) define a representative sample of products, leveraging product data from the large number of participating companies, and (2) calibrate an A to E scoring scale. Furthermore, cosmetic products include a broad array of diverse ingredients within each product segment. Gathering data for such a large number of ingredients is challenging, so we developed read-across and other approaches to avoid a no data, no impact situation, but this led to varied levels of data representativeness among ingredients.

The PEF initiative provides precise instructions for dealing with data collection and data quality. At this stage of EBS method development, strict adherence to these instructions was not achievable for the large ingredient portfolio in scope, and this will be addressed in the future. In the interim, and following the principles of PEF, we developed a numeric indicator to understand the representativeness of our ingredient data characterizing production and end-of-life impacts. For packaging, there is less material diversity and no pathway in the EBS method to score a product that lacks specific. Therefore the indicator characterizes ingredient data representativeness only.

This indicator was used to select the products to include when calibrating the A to E scoring scale, ensuring only inclusion of products with footprint values underpinned by a high proportion of representative data. This approach is key to ensure that meaningful consumer-facing scores are communicated and to prioritize future data development to improve representativeness.

Disclaimer/Disclosure: The EcoBeautyScore initiative started as a consortium in 2021 and turned into an association (under the Belgium Law) in 2024.

5.09.P-We454 Integrating Safe and Sustainable by Design (SSbD) Principles into the Battery Passport: Necessities and Benefits

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The battery passport represents a transformative initiative for ensuring transparency and sustainability in the battery value chain. However, while numerous efforts have been made to establish this tool, integrating Safe and Sustainable by Design (SSbD) principles has yet to gain traction. This omission is partly due to the absence of legislative requirements. Nevertheless, the SSbD framework, with its holistic approach to safety, environmental, economic, and social dimensions, has the potential to enhance sustainability aspects critical to battery development usage, recycling and reuse. Its adoption could also promote broader dissemination and comprehension across the value chain.

To address this gap, work performed analyzed existing battery passport initiatives to identify the

sustainability metrics they incorporate and pinpoint areas for improvement. Building on these insights, it is proposed specific indicators aligned with the SSbD framework that address current deficiencies. Approach developed is grounded in a prioritization and scoring system designed to quantify SSbD performance focused in areas covered by the battery passport. This system integrates, as a keystone, stakeholder perspectives, ensuring that the proposed metrics are both practical and aligned with the expectations of key actors in the battery ecosystem.

Although results from this case study are pending, preliminary findings suggest that the SSbD-based scoring system aligns well with legislative requirements such as the EU Battery Regulation and the proposed sustainability criteria for batteries. By addressing both current and anticipated regulatory frameworks, the SSbD framework and linked developed system offers a robust, forward-thinking solution for the integration of sustainability and safety considerations into the battery passport.

Hence, study has allowed to highlights the relevance of embedding SSbD principles into such emerging initiative. It underscores how these principles can bridge gaps in existing methodologies, foster value chain alignment, and enhance the overall safety and sustainability of battery technologies. Such advancements are crucial for enabling the transition to a more sustainable and secure energy future.

5.09.P-We455 Assessing the Environmental Footprint of the Dutch Healthcare Sector – A Hybrid Methodology

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Within the global efforts towards a sustainable society, monitoring national footprints is a central tool to track sustainability targets. In the Netherlands, these targets have been set on a sectoral level to improve decision-making, since different economic sectors impact the environment in distinctive ways. In this line, the healthcare sector has committed to monitor its own environmental footprint to track its contribution towards climate, biodiversity and circular economy goals. A first calculation showing that healthcare is responsible for 7.3% of the national carbon footprint reflects the urgency for mitigation action within the sector.

While environmental footprints for healthcare sectors mostly result from input-output analyses (IOA), the Dutch healthcare sector s will be periodically calculated using a hybrid methodology combining the completeness of an IOA with the specificity and actuality of life cycle assessments (LCA) to the largest extend possible. In comparison to a classic IOA, this hybridization allows for a further specification of the results, whether between sub-sectors (e.g., hospitals, residential care,) or between products/services that are aggregated within IOA databases (e.g., medical appliances, pharmaceuticals,), making the results more actionable. It also allows for more actual data from healthcare institutions and related industries to be incorporated so that mitigation efforts can be better reflected in the results.

The hybrid methodology results from a review of the available multi-regional input-output (MRIO) databases and from a thorough inventory of the existing data sources within the Netherlands. It will be applied iteratively to respond to data needs for tracking environmental goals and fulfilling legal obligations applying to the sector (e.g., from the corporate sustainability reporting directive (CSRD)) while considering practical limitations, such as potential administrative burden linked to data collection. The first iteration will combine data from the PBL-FIGARO MRIO database, expenditure data from healthcare institutions and from consumption of medical products, as well as transport, energy usage, and water usage data collected within the sector, and waste production and management data collected nationally. The environmental impacts that will be calculated are greenhouse gas emissions, resource depletion, land use, water consumption, and waste production.

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5.09.P-We456 Regional Variability in the Carbon Footprint of Canadians: Insights from OpenIO-Canada

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Decarbonization goals demand precise and regionally specific assessments of greenhouse gas (GHG) emissions to support effective climate mitigation policies. This study introduces OpenIO-Canada, a multi-regional environmentally extended input-output database with capital endogenization, developed to assess

the consumption-based carbon footprint (CBCF) of Canadian provinces and territories (P&Ts) in 2019. Unlike traditional territorial-based assessments, CBCFs attribute emissions to final consumers, accounting for the production and transportation of goods and services across regions and internationally.

Our analysis highlights significant disparities among Canadian P&Ts. While the average Canadian emits 18 tCO₂eq. per year, per capita CBCFs range from 14.5 tCO₂eq. in Quebec to 38 tCO₂eq. in the Northwest Territories, driven by differences in population density, consumption patterns, energy mix, and infrastructure needs. Housing, transport, and food collectively account for 69% of Canada's CBCF, with transport emissions dominated by direct vehicle use, housing emissions linked to energy consumption and construction, and food-related emissions stemming from agricultural production, particularly meat and dairy.

Notably, capital formation representing infrastructure and durable goods contributes 19% of the national CBCF, underscoring the importance of accounting for these emissions in climate strategies. Regional energy production methods significantly influence carbon intensity, with provinces like Alberta and Saskatchewan displaying higher footprints due to fossil fuel-based electricity, compared to provinces with cleaner energy mixes such as Quebec and British Columbia.

The findings offer actionable insights for stakeholders. Policymakers can leverage these results to prioritize decarbonization strategies tailored to regional contexts, focusing on reducing emissions in housing through energy efficiency, promoting sustainable transport, and shifting to lower-carbon diets. The integration of capital endogenization enhances the accuracy of CBCF assessments, emphasizing the need for sustainable infrastructure investments. OpenIO-Canada's detailed regionalization provides a robust foundation for advancing Canada's transition to net-zero emissions by 2050.

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5.09.P-We457 Life Cycle Assessment of Crops in India

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In India, the agricultural sector is undergoing rapid modernization to meet the food demands of a growing population. However, this modernization has negative impacts on the ecosystem, human health, and resources due to the extensive use of agrochemicals and emission-intensive farming practices. It is therefore crucial to assess the sustainability of agriculture in India. This paper presents the first-ever impact assessment of the cultivation of 21 commonly grown crops in India, focusing on their production and emissions. The study identifies the crops with the highest impact on various parameters, such as rice, sugarcane, wheat, and banana. For instance, coconut cultivation is found to contribute significantly to global warming, while potato and sugarcane have a greater impact on water and ozone depletion, respectively. The study recommends improvements in farming practices to reduce emissions and promote sustainability. Additionally, the paper evaluates the connection between these 18 impact indices and the 17 sustainable development goals (SDGs) related to agriculture. This assessment helps identify the appropriate indices to measure agricultural sustainability and highlights areas for improvement or the need for new indices. By considering these findings, India can work towards achieving the SDGs while minimizing environmental impact and ensuring socio-economic aspects are not compromised in crop production and agricultural systems.

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5.09.P-We458 Packaging Choices and Their Role in Reducing Product Loss and CO₂ Impact of Moisturizing Products: Insights for PEF Methodology and Sustainable Design

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The primary function of packaging is to protect the packaged good, ensuring its integrity and usability throughout its lifecycle. Improper packaging can lead to product loss, increased environmental burdens, and economic inefficiencies.

A benchmarking study of 124 cosmetic products on the Austrian, German, and Swiss markets revealed that viscous products are prone to leave significant residues in packaging such as 26.65% for hand creams in pump dispensers resulting in considerable waste. Life cycle assessment (LCA) comparisons reveal that while packaging materials differ in environmental impact, the filling product typically has a significantly higher CO₂ impact than its packaging. Thus, selecting the correct packaging is critical to minimise product loss and its associated environmental consequences.

This study focuses on climate change impacts of packaging formats and product loss due to incomplete emptiability. Other loss factors, such as incorrect storage or handling, are acknowledged but not included due to limited data availability. The research calculates the CO₂ impacts of various moisturiser packaging formats across their lifecycle, including squeeze bottles, tubes, pump dispensers, jars, airless pump dispensers, and pouches. A single moisturising product formulation is used to standardise LCA comparisons. The LCA study uses the Circular Footprint Formula, as outlined in the Product Environmental Footprint method.

The study will test two hypotheses: (1) for viscous moisturising products, the CO₂ impact of the product itself exceeds that of its packaging by at least 80% throughout its lifecycle, and (2) the ranking of CO₂ impacts across packaging formats shifts when the CO₂ impacts of moisturiser residue are included, as the total impacts (packaging + residue) alter the relative environmental performance of each format. These findings aim to advance sustainable packaging design by identifying residue thresholds that balance environmental and economic trade-offs.

By linking product loss to LCA outcomes, this research underscores the need for packaging designs that minimise residue, reducing both environmental and consumer costs. These findings also lay the groundwork for developing Product Environmental Footprint Category Rules, highlighting the role of packaging functionality such as product protection and emptiability often overlooked in traditional LCA comparisons.

5.09.P-We459 How much of the Smart Grid Have We Mapped? A Review of Their Environmental Coverage

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With the increasing electrification of different sectors, smart grids (SG) are considered a critical technology supporting decarbonization in our societies. SG involves a massive deployment of devices to gather and transport information and act upon the grid remotely or automatically. We want to know how much of this grid has been environmentally assessed, what needs to be done, and which claims have been made until now.

The assessment started with an exhaustive bibliometric review following the PRISMA methodology, combining a structured and non-structured literature search. We considered work that looks at infrastructure needs, conceptual frameworks, and visions of SG and its technologies, as well as environmental assessments.

Most of the reviewed work ensures that SG will bring energy and greenhouse gas (GHG) savings. This is justified through increasing energy efficiency, although some studies point out that rebound effects could jeopardize these savings. Studies have only considered the end of the distribution lines, considering smart meters and Home Energy Management Systems, leaving the whole transmission and most of the distribution lines unattended.

Due to SG's complexity, the type and quantity of devices used in the two grids will not be the same, making it impossible to determine a fixed set of devices to model SGs. Nevertheless, we believe there is an over-optimistic vision of SG based on a broad vision of the concept and a partial assessment. We

hypothesize that deploying the infrastructure may entail emissions beyond GHG that energy savings might be unable to balance. Therefore, a broader perspective of the SG infrastructure should be captured, and further environmental analyses should be conducted on the distribution and transmission infrastructure.

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5.09.P-We460 Environmental Sustainability of Conventional and Electric Bicycles: A Review

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In 2022, the transport sector accounted for 29 % of the European Union's (EU) greenhouse gas (GHG) emissions, making it one of the major sources of these emissions in EU. The EU's primary objective in combating climate change is to achieve carbon neutrality by 2050, positioning itself as the world's first carbon-neutral continent. Transitioning to low-emission transport modes has been identified as a key strategy to reach this target. Bicycles, particularly electric bicycles, have become increasingly popular as a strategy for reducing reliance on fossil-fuel-powered vehicles. The environmental impacts of bicycles, conventional and electric bicycles, have been evaluated with the Life Cycle Assessment (LCA) methodology. However, methodological choices and assumptions have led to different LCA results. This study conducts a systematic review of LCA-based research on the environmental performance of conventional and electric bicycles to identify and understand, in a quantitative analysis, how different methodological choices influence global warming (GW) results. The literature review has been conducted using electronic databases (Scopus and Web of Science) for LCA-based studies published in peer-reviewed journals. A final sample of 28 papers published in peer-reviewed journals, was reviewed, based on a thorough analysis of methodological choices, including functional unit, the system boundaries, and the most evaluated impact categories.

The results show that most case studies (89 %) applied a cradle-to-grave approach, while cradle-to-gate was considered in 7 % and cradle-to-user in 4 % of the case study papers. High variability was observed for GW, ranging from 0.0015 to 0.3150 kg CO₂eq/pkm for conventional bicycles and 0.0160 to 0.1673 kg CO₂eq/pkm for electric bicycles. The higher results for both bicycle types relate to the shared bicycle systems. The results show that conventional shared bicycles present higher GW impact values than shared electric bicycles, primarily due to the overall shorter lifespan distance of conventional bicycles compared to the longer lifespan distance of electric bicycles.

The EoL stage of electric bicycles still needs more detailed data. Future research should harmonise the methodological choices, and explore EoL waste treatment, especially for batteries (Lead-acid and Lithium).

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5.09.P-We461 Providing a Science-Based and Harmonized Environmental Scoring Scheme for Cosmetics: The EcoBeautyScore

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The EcoBeautyScore (EBS) Association*, comprising over 70 global cosmetic companies and professional associations, has developed a unified environmental footprint measurement and scoring system for cosmetic products over the past three years. This system is based on Life Cycle Assessment (LCA), recognized by the European Commission (EC) as the most effective method for assessing and comparing the environmental footprint of products and services. The LCA methodology builds on the

Product Environmental Footprint (PEF) guideline initiated by the EC in 2013. Moreover, the method was enhanced to suit the specificities of cosmetics. This communication is dedicated to presenting the overall EBS footprint and scoring methodology.

The EBS footprint methodology aligns with the key PEF principles, assessing 16 midpoint impact categories across a full cosmetic product's lifecycle. Products are evaluated based on the same functional unit, using a data hierarchy to derive usage doses for cosmetic technologies. Moreover, to address limitations concerning robustness and data coverage in the PEF-prescribed USEtox 2.1 model for freshwater ecotoxicity, an adapted method was implemented using the Most Sensitive Species-HC5 (hazardous concentration for 5% of species) approach.

A common database for production and end-of-life impacts of standard raw materials was created, prioritizing prevalent and impactful ones, complemented by a comprehensive data strategy. Significant enhancements in data coverage and quality for the end-of-life ecotoxicity freshwater database were achieved by developing new characterization factors. Additionally, incorrect and obsolete factors were replaced to enhance applicability of the USEtox model for cosmetics.

To make PEF outcomes clear to consumers, a product segmentation approach is used, comparing only products with the same primary cosmetic benefit. A portfolio assessment combined with a representative sampling defines environmental performance classes from A to E per segment.

Tested on over 1,900 products, results show that for rinsed-off products like Hair Wash, the use phase is a major PEF contributor, followed by ingredient production and end-of-life. For Face Care products, ingredients are also a significant contributor, but they show no use phase impacts, and packaging production has a more significant impact.

* The EcoBeautyScore initiative started as a consortium in 2021 and turned into an association (under the Belgium Law) in 2024.

5.09.P-We462 Using LCA to Support Decision-Making in Water Management: Conventional vs. Desalination Strategies in Sicily

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The Life Cycle Assessment (LCA) framework has become an important tool for quantifying environmental sustainability and guiding decision-making in policy and industry. This study applies LCA to evaluate water management strategies in Sicily, Italy, a region severely affected by climate change and water scarcity.

Rising global temperatures and extreme weather events have drastically reduced water availability, with national water resources declining by 20% during 1991-2020 compared to the 1921-1950 average. With its aging infrastructure and significant resource losses (45% in distribution networks), Sicily is among the most impacted regions.

This research focuses on comparing the environmental performances of three water production methods: conventional sources, seawater desalination, and brackish water desalination from central Sicilian aquifers.

Groundwater, currently supplying 42% of distributed water, has been overexploited, leading to critical aquifer depletion and chemical contamination.

Desalination, though underutilized in Italy, offers a promising alternative. However, its adoption faces barriers such as limited R&D investment, high energy demand, and complex permitting processes. Comparatively, countries like Spain have successfully integrated desalination into resilient and diversified water systems.

Recent legislative measures, such as the Italian Drought Decree (Decree-Law No. 39 of April 14, 2023), aim to streamline the development of small desalination plants (<50,000 m³/day), but comprehensive feasibility studies are still required to assess their environmental and economic viability.

The LCA framework in this study is designed to quantify the environmental impacts of these methods and support policymakers. Particular attention is given to methodological choices, such as system boundaries and impact categories, to ensure robust and actionable results.

Situated in a water-stressed region, this research highlights the importance of integrating innovative water technologies into sustainable and resilient management strategies, aiming to bridge the gap between environmental analysis and practical implementation.

5.09.P-We463 Enhancing Environmental Impact Assessment: A Multi-Lens Approach to Sustainable Product Design for Cosmetics & Personal Care Products

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In the Cosmetics and Personal Care sectors, higher environmental impacts typically occur during the production of raw materials used to create formulations and packaging, and their impact after consumer disposal. To reduce the environmental impact of such products, integrating sustainability into the product design process is therefore becoming increasingly important. The development of sustainable design tools, built on scientific principles is key to guide product development teams.

Life Cycle Assessment (LCA) is often considered the gold standard for measuring product environmental impact, but it is only one lens to measure sustainability through and has limitations for certain product types. An assessment of product environmental impact can be strengthened by combining LCA with additional methodologies that highlight other important factors (e.g., unforeseen persistence-related impacts of ingredients and materials). Leveraging a multi-lens approach can create a decision-making framework which prevents product developers from making unintended tradeoffs when designing for sustainability.

The Sustainable Innovation Profiler (SIP) is a patent pending, sustainable design tool that enables product developers to compare a new product's environmental impact with that of a baseline during the prototyping process and assess the holistic environmental impact of products in the Cosmetics and Personal Care sectors. The SIP tool includes three separate methodologies. The first is an LCA-based method inspired by the European Commission's Product Environmental Footprint (PEF) method, assessing a product's cradle to grave environmental impact across 16 key indicators. A second method evaluates the environmental impact of the product's formulation through a different lens. This method has the peer reviewed and patented Global Aquatic Ingredient Assessment (GAIA) at its core with scoring modifications for: (1) ingredients with potential emerging concerns and (2) ingredients sourced from a renewable origin. Finally, a third method measures the sustainability of the product's packaging using a framework which emphasizes the importance of designing packaging for recyclability, increasing the use of recycled materials and using materials efficiently.

This poster introduces the tool and its holistic approach to assessing product environmental impact and features a case study on the application of this framework for a product from the Cosmetics and Personal Care sector.

5.09.P-We464 Enhancing Sustainability in Biotechnology: A Comparative Life Cycle Assessment of Single-Use Assemblies

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The Life Science business of Merck KGaA, Darmstadt, Germany, offers a diverse portfolio of over 300,000 products essential for research and development in biotechnology and pharmaceuticals. In July 2023, we implemented a portfolio sustainability assessment (PSA) approach, based on the World Business Council for Sustainable Development (WBCSD) standards, to facilitate our sustainable portfolio transformation. This initiative integrates hazard screening, regulatory trends, and sustainability performance, aligning with the Safe and Sustainable by Design (SSbD) framework. The inherent complexity of Merck's chemical portfolio poses challenges in comprehensively applying ISO-compliant Life Cycle Assessment (LCA), necessitating a practical approach for communicating results to non-experts and for reporting on environmental sustainability.

In this study, we conducted a comparative LCA of two single-use assemblies, each utilizing different films. One assembly features an improved film with enhanced bag strength, improved durability, and leak resistance through a novel strength layer reinforced by woven nylon. The study adhered to ISO 14040/44 standards using the EF method 3.1. An independent critical review ensured compliance with these standards, reinforcing the study's reliability and trustworthiness. For this, we engaged experts for the critical review panel, ensuring comprehensive coverage of necessary expertise. Challenges primarily arose in data collection due to the novelty of the process and significant data gaps from incomplete supplier information. To address these, we employed AI tools to fill data gaps for mass flows and energy demands. The motivation behind this extensive effort was to produce a credible and trustworthy study that fosters customer confidence by demonstrating the environmental performance of the novel and improved film for challenging single-use applications. This contribution discusses the challenges encountered in designing and executing the LCA, particularly in terms of methodology, data management, and communication of results to non-experts. It underscores the critical role of LCAs in decision support and reporting, highlighting the need for standardization and harmonization across sectors to enhance comparability and

effectiveness. Our findings contribute to the ongoing dialogue around the strengths and limitations of LCA frameworks, identifying future needs for methodological advancements in the Life Science sector.

5.09.P-We465 Quantification of Potential Impacts in the End-of-Life Phase of Construction Products in the EPD

Tatiana Trecakova and Vladimir Koci, University of Chemistry and Technology Prague (UTC), Czech Republic

EPDs (Environmental Product Declarations) are becoming a crucial tool in corporate sustainability and the entire supply chain. EPDs for construction products are prepared in accordance with EN 15804:2012+A2:2019+AC:2021 and the PCR for construction products, issued by EPD system operators. The aim of the Product Category Rules (PCR) is to provide verifiable and consistent data for LCA-based EPDs.

Construction products must declare modules A1-A3 (production phases), C1-C4 (end-of-life phases), and D (benefits and loads beyond the system boundary). Neither the PCR nor the standard provides specific requirements for phases C1-C4, which may lead to different approaches in quantifying potential environmental impacts. For phases C1-C4, which concern the end-of-life stage, it is required to consider impacts in a defined scenario.

In this study, different approaches for defining the scenario for phases C1-C4 are discussed. These approaches may depend on the region where the product will reach its end-of-life, the availability of data on different treatment options (by region, by time), and assumptions regarding consumer behavior, etc. The different approaches for the C1-C4 phases have been applied, analyzed, and evaluated, and the weak points have been identified using the example of a selected construction product.

A more accurate quantification of environmental impacts in the end-of-life phases would facilitate higher transparency in decision-making mechanisms and the enforcement of a circular economy.

5.09.P-We466 Organizational Climate Impact Accounting - Accounting for Actions That Matter: Supporting Sustainable Consumption of Buildings

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This study proposes a new framework for carbon accounting, referred to as climate impact accounting. This new framework is focused on providing evidence-based decision support to reach current climate targets. Therefore, the study mainly focuses on cause-effect relationships, which ensures avoiding burden-shifting from one organisation to another and to avoid suboptimal decision that introduces additional GHG emissions at other points, in a system wide perspective. The study aims to account for potential impacts rather than to account for the share of global burdens belonging to the organisation as to provide better evidence-based decision support for a decision. Consequential modelling (cLCA) is used as the underlying methodology for this study as it is acknowledged to provide optimal decision support through its use of marginal suppliers among other elements. The framework is applied to the organisation; Aalborg University to evaluate it and its functionality and applicability, and is based on an extensive consensus project for organisational footprints supported by Danish Universities (DKUNI). The paper therefore also presents important improvements to current carbon accounting guidelines such as the GHG Protocol (GHGP) and ISO standards on LCA. The strength of the approach is exemplified by addressing how to apply improved impact accounting of the use of buildings. The GHGP argues that emissions from buildings should only be accounted for once construction is completed. Therefore, if a building undergoes no maintenance and consumes no heating or electricity in a given year, it would be considered climate neutral. However, the proposed climate impact accounting method suggests that there is still an impact associated with the occupation of buildings. Occupying a building effectively removes it from the available market, which will drive an increase in the construction of new buildings from a system-wide perspective. Conversely, reducing the building stock at AAU would free up more space on the market, thus displacing the need for new construction. As a result, even a building built many years ago and with no maintenance or operational activity still has a climate impact as long as it remains occupied. This concept is similar to the principle applied to indirect land-use change (iLUC) in EXIOBASE.

5.09.P-We467 Life-Cycle Based Assessment of Green Laboratory Practices – A Case Study From an Austrian Institution of Higher Education

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Recently, scientists have started developing an increasing awareness of the contribution of scientific practices to the global environmental crisis. Across disciplines grassroots movements are emerging, which promote improvements of the environmental footprint of scientific activities.

In institutions of higher education (IHE), experimental laboratories for chemistry and life sciences, so-

called wet labs, usually have particularly high unwanted environmental impacts. Therefore, a growing body of publications, programs and frameworks is promoting so-called green practices for these laboratories. Recommendations for such practices are very diverse, including e.g. guidelines for the handling of energy-consuming equipment like fume hoods and cold storage devices, establishment of green procurement, as well as training of researchers and students to foster waste prevention and separation. Efforts to establish green laboratory practices, however, often face severe resistance from laboratory staff and management alike, as some of them require behavioral changes and significant organizational efforts to implement.

One way of addressing such objections is data-based communication of environmental impacts and the potential of green lab practices to improve these impacts. Thorough assessment of individual practices also allows prioritization of measures with high environmental benefits. Thus, informed decision-making and effective improvement of environmental impacts is enabled within institutions.

We present data from a case study conducted at the chemistry and life science laboratories of the UAS Technikum Wien, an IHE in Austria. Based on on-site waste analyses and electricity measurements, selected green laboratory practices promoted in literature and by green lab initiatives are evaluated. Life cycle assessment is used to evaluate the environmental benefits of reduced use of consumables, as well as collection and separation of single-use bulk plastics for recycling. Moreover, reduction potential of the laboratories electricity demand is assessed.

By quantifying the potential impacts of green laboratory practices, the presented work contributes to data-based discussion and decision-making within the UAS Technikum Wien. Thus, implementation of such practices can be promoted more effectively.

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5.10 Life Cycle Assessment of Waste and Waste Management Systems for Safe and Sustainable Futures

5.10.T-01 A Global Life Cycle Inventory Model for Urban Wastewater Treatment and Sanitation *Ivan Muñoz, 2.-0 LCA consultants, Spain*

We present WW LCI v5, a life cycle inventory (LCI) model developed during the last 10 years by 2.-0 LCA consultants. WW LCI covers the entire supply chain for wastewater management, namely wastewater collection and direct discharges, centralized wastewater treatment with different plant configurations and treatment levels, sludge treatment with and without anaerobic digestion and cogeneration with biogas, and sludge disposal (composting, agriculture, controlled/uncontrolled landfill, incineration), as well as wastewater reuse in agriculture. The model requires the user to define the composition of the wastewater discharge. This can be done at two levels or tiers: tier 1 where the user only knows some generic descriptors, such as chemical oxygen demand, while in the more advanced tier 2 the user can identify specific chemicals present in the wastewater (e.g. ethanol, sodium chloride).

The model is highly parameterized with more than 200 parameters. Also, a key feature is the country database, containing statistics on wastewater collection, treatment levels, sludge treatment and disposal, climate (temperatures, rainfall) for 101 countries and 2 regions (EU, GLO). A drop-down menu allows the user to automatically load a country scenario, thus accounting for geographical differences with just one click.

The model is programmed in Excel and links to the ecoinvent database. LCIs obtained by the user can then be easily exported to both SimaPro and Gabi software, in order to conduct impact assessment calculations.

We will show examples of using the model for different wastewater compositions in different geographies, the differences between tier 1 and tier 2 modelling, the importance of methane emissions in this area and of accounting for credits associated to the recovery of materials and energy.

WW LCI is the successful story of a crowdfunded project with a current community of 29 users, which are allowed access through a one-off fee. This allows 2.-0 LCA consultants to cover maintenance and further development costs. The model still has limitations, such as countries not yet covered, and the focus on urban wastewater, which limits to some extent its use to model industrial wastewater treatment. Yet, to our knowledge it is the most versatile and complete model for LCA practitioners willing to go a step further in addressing the life cycle impacts of wastewater discharges.

5.10.T-02 Reducing the Climate Impact of Residual Waste Treatment: A Comparative LCA of Carbon Management Strategies in a German Context

Sarah Schmidt and David Laner, University of Kassel, Germany

Achieving climate neutrality targets necessitates the evolution of MSW management concepts and treatment technologies. The retrofitting of MSWI with carbon capture and storage (MSWI-CCS) technologies and the recovery of materials from residual waste in material recovery facilities (MRF) are discussed as key carbon management strategies in MSW management in Europe. Identifying the long-term environmentally optimal waste management concept is crucial, as decisions in waste management often concern waste technologies with related infrastructure operating for 30 years or more. However, the evaluation of long-term waste management concepts is challenged by anticipated societal transformations over the next decades. Using the city of Kassel (Germany) as a case study, we perform a life cycle assessment to evaluate and compare MRF and MSWI-CCS as strategies for the reduction of GHG emissions associated with residual waste management. Sensitivity scenarios are employed to examine the impact of varying constraints, considering changes in waste inputs, material and energy systems, and substitution choices. Moreover, uncertainty analysis is applied to quantify the variability of GHG emissions linked to residual waste treatment resulting from parameter uncertainty.

The analysis revealed that residual waste treatment in Kassel in 2022 resulted in the direct emission of 857 kg CO₂ per Mg of wet residual waste, with approximately 31% fossil CO₂. Considering environmental savings linked to the recovery of energy and materials net GHG savings of 141 kg CO₂-eq. per Mg wet residual waste were achieved. Carbon management strategies show potential to reduce direct fossil CO₂ emissions by 27% (MRF) or 90% (MSWI-CCS) and to increase the net GHG savings of residual waste management by factor 1.6 (MRF) or 2.6 (MSWI-CCS). The comparative performance of MRF strongly depends on waste compositions, being preferable against MSWI-CCS for a residual waste with a high recyclables content. Overall, S-MSWI-CCS exhibits the highest potential for GHG reduction, outperforming S-MRF and S-MSWI under various conditions because of its resilience against variations in waste composition, energy and material systems, and substitution choices. In addition to offering practical guidance for residual waste management, this study underlines the importance of future-oriented decision-making in residual waste management and highlights the need for further research in prospective waste system modelling.

5.10.T-03 Modelling Metals Recycling With a Dynamic Lca Approach: A Methodological Discussion Based on a Real-World Case Study

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This study explores methodological approaches for modeling metals recycling using a Life Cycle Assessment (LCA) approach, drawing insights from both the ALCHIMIA and GRINS projects. The ALCHIMIA project focuses on the steel industry and utilizes a dynamic LCA approach that evaluates the environmental impacts of steelmaking using scrap materials. This study aims to provide a detailed comparison of three methods for modelling recycling in LCA: cut-off, system expansion, and the Circular Footprint Formula (CFF). These methods differ in how they allocate environmental burdens to recycled materials. In the cut-off method, recyclable materials are considered burden-free, with only the impacts from the recycling process, including energy, virgin materials, and transportations. The system expansion approach, also known as the substitution or avoided burden method, allocates a share of the previous lifecycle impacts of the material to the recycled product and subtracts the impacts from avoided production of primary materials. The CFF serves as an intermediate approach, providing a more balanced allocation of environmental burdens. The study employs a heat-by-heat dynamic LCA approach, in which Life Cycle Inventory (LCI) data vary depending on the specific steel product being produced and the composition of the scrap inputs, offering a granular understanding of recycling impacts in the steel industry. Data from a real-world steel plant were collected and modeled using all three approaches across eight steel families. Results showed that the cut-off approach, while providing less variable result, is more suitable for general benchmarking and baseline evaluations. On the other hand, the system expansion approach is better for understanding the impacts of specific products or the dynamic nature of the scrap mix. The CFF approach offers a flexible middle ground between the two, allowing for a trade-off between the advantages and disadvantages of the cut-off and system expansion methods. These findings are expected to guide future LCA studies, particularly in the context of the GRINS project, which applies similar methodologies to the aluminum sector. Further research could extend these insights to other recycling processes in the metal industry, enhancing the understanding of environmental impacts and supporting the decision making process.

5.10.T-04 Does Machine Learning Pay Off in Terms of Environmental Impacts? Insights from the Waste Management Sector

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The European Commission's Circular Economy Action Plan (CEAP) established the circular economy as a key element to achieve the European Green Deal's 2050 net-zero emissions target. For effective recycling, the waste management sector must adopt efficient waste collection, transportation, and sorting methods. The increasing volume and diversity of materials in waste streams, coupled with demands for low-emission secondary resources, call for new and innovative approaches. Artificial intelligence (AI), particularly machine learning (ML), offers promising solutions for optimizing these processes, such as improving waste sorting with advanced pattern recognition. However, it is essential to assess whether the environmental benefits of ML outweigh its potential impacts, particularly its energy consumption.

Recent research highlighted the need to evaluate AI's environmental effects holistically, not just focusing on operational energy use but also considering the other impacts, using Life Cycle Assessment (LCA) methodology. Therefore, our study applied LCA to determine if ML's process efficiencies yield net environmental benefits within waste management. The study assessed four use cases in the plastics recycling chain, covering collection and sorting processes of plastic packaging waste.

The study's objectives were threefold: (i) to compile primary datasets on waste management activities through industry collaboration, addressing data gaps that are often filled with generic data; (ii) to assess the environmental impact of four ML technologies using LCA methodology; and (iii) to evaluate the net environmental benefit (or burden) of implementing ML in waste management.

Four use cases were analyzed: intelligent route optimization for waste collection (Use Case 1), inventory optimization to reduce empty trips (Use Case 2), and two additional ML applications for improving sorting efficiency. The LCAs followed ISO 14040/14044 methodology, with a consistent functional unit of treating one ton of post-consumer plastic waste and considered global warming potential, energy demand, and resource depletion impacts. The study results provide valuable primary datasets and offer novel insights into the environmental trade-offs of AI applications in waste management.

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5.10.P-We468 Bayesian Belief Networks As a Tool to Inform on Plastic Clean-Up Technologies Net Benefits

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To collect and remove legacy plastic, which accumulates in the environment, new technological interventions are being deployed. These so-called plastic clean-up technologies can be installed in inland waterways, such as rivers and estuaries, where they can avoid the further spread of plastic to marine environments. There is however a lack of standardization in reporting plastic removal and unwanted biological bycatch since empirical data is scarce. The goal of this work was to develop, train, and validate Bayesian Belief Networks for plastic clean-up technologies. We propose to use these probabilistic models as a tool to assess the proportion of plastic and biota collection and removal by different clean-up mechanisms. The models can support stakeholders in deploying a clean-up technology by providing the proportion of plastic and biota collected depending on the technology, plastic pollution, and environmental conditions. To train, test, and validate the models, we used a set of experimental data collected in a current flume system run under controlled conditions. To develop the models, we have identified six variables in relation to three key aspects based on their ability to determine plastic removal and the biological bycatch. The type of clean-up mechanisms used (an air bubble curtain, two floating booms with a wheel) is a first key aspect. The intrinsic characteristics of the plastic and biota items (e.g., size, shape) as well as the number of items present have been identified as a second important set of key aspects. Finally, the hydraulic condition (flow velocity) of the waterway in which a mechanism is deployed is also incorporated. Based on the model with the highest technical reliability, simulations will be made to provide relevant scenarios and to evaluate the effect of the variables (e.g., flow velocity) on plastic and biota collection and removal.

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5.10.P-We480 Safe and Sustainable Recycling of Solar Panels: A Scenario Study

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To assess and stimulate safe and sustainable recycling the National Institute of Public Health and the Environment (RIVM) developed a screening methodology to assess the circularity, environmental impact and safety of recycling of waste flows. This method was applied on the case of recycling of End-of-Life solar panels. It is expected that the first generation of solar panels will start to be taken out of use in large numbers in five years time. Various technologies to recycle solar panels are being developed.

The aim of this study was to investigate which recycling options appear to be feasible, how environmentally friendly they are and to learn how the screening methodology can help to make a comparison. We compared four future scenarios to the current situation (the baseline), in which no raw materials from End-of-Life solar panels are recovered, but are shredded and processed for a low-grade applications like road foundation. Three screening modules were applied for quantifying the circularity, environmental impact and the safety due to the presence or emission of substances of concern (SoC). Our analysis shows that all future options are more circular and have a lower environmental impact than the baseline. One scenario scores best on climate change (CO₂-eq.), but all are much lower than the baseline. One of reasons is that it costs more energy to process new raw materials into solar panels than to use recycled raw materials. The option in which glass is recycled into new solar panel glass and the silicon can be recycled to high grade silicon, has also the highest value for the circularity indicator 'efficiency'. In the recycling process, attention should be paid to hazardous substances in solar panels: lead, antimony and per- and polyfluoroalkyl substances (PFAS). Lead is contained in soldering materials and antimony is added to make the glass brighter. The backsheets contain fluoropolymers, as a result of which PFAS can be released when they are incinerated. This can lead to human and environmental exposure, depending on how substances are released.

Based on this study we advised the national government to stimulate specific technological developments that enable high-grade recycling of solar panels. We also recommend to stimulate design for recycling. For example developing other encapsulant materials between the glass and the backsheet enables easier dismantling. It is also important to minimise the use of hazardous substances lead and PFAS.

5.10.P-We481 Bottom-Up, Dynamic Probabilistic Material Flow Analysis of Japanese Plastic Flows as an Initial Step to Understand the Chemical Additives Flows and Stocks

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The global production of secondary plastics has increased more than tenfold in the past two decades, creating a strong backflow from end-of-life (EoL) products to manufacturing. Secondary materials can substitute pristine resins in manufacturing, and therefore mitigate many of the related environmental impacts. However, the associated risks of secondary materials, particularly from the additives that persist through recycling processes, are not yet fully understood. Tracking the flows of chemicals in secondary materials requires high-resolution data on plastic flows. As a first step of achieving so, this study presents a dynamic probabilistic material flow analysis of plastic flows, with high granularity at the EoL stage. Japan was selected as a case study, due to the availability of comprehensive data. We compiled the production, trade, and consumption data for eight major types of commercial polymers for the period from 1950 to 2023. The amounts were allocated to 48 product categories following a bottom-up approach based on the annual sales data and the polymer composition of these products. Using the consumption data and lifetime distributions, we estimated waste generation for individual product categories. Twenty-three collection pathways were differentiated across general waste collection, industrial waste collection and sorted collection for specific product categories that are regulated under existing waste laws and/or managed by various industrial associations. The inputs and outputs of mechanical recycling were modeled accordingly. Our results show a significant increase in recycling activity following the establishment of respective recycling laws. However, the absolute quantities of secondary plastics remain relatively low compared to those that were sent to energy recovery and landfill. Secondary materials primarily originate from containers and packaging, electronics, and motor vehicles. These findings provide initial insights into the types of secondary polymers and chemicals likely present therein, forming a basis for future quantification of the flows of chemical additives and their environmental and human health impacts.

5.10.P Life Cycle Assessment of Waste and Waste Management Systems for Safe and Sustainable Futures

5.10.P-We468 Bayesian Belief Networks as a tool to inform on plastic clean-up technologies net benefits

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To collect and remove legacy plastic, which accumulates in the environment, new technological interventions are being deployed. These so-called plastic clean-up technologies can be installed in inland waterways, such as rivers and estuaries, where they can avoid the further spread of plastic to marine environments. There is however a lack of standardization in reporting plastic removal and unwanted biological bycatch since empirical data is scarce. The goal of this work was to develop, train, and validate Bayesian Belief Networks for plastic clean-up technologies. We propose to use these probabilistic models as a tool to assess the proportion of plastic and biota collection and removal by different clean-up mechanisms. The models can support stakeholders in deploying a clean-up technology by providing the proportion of plastic and biota collected depending on the technology, plastic pollution, and environmental conditions. To train, test, and validate the models, we used a set of experimental data collected in a current flume system run under controlled conditions. To develop the models, we have identified six variables in relation to three key aspects based on their ability to determine plastic removal and the biological bycatch. The type of clean-up mechanisms used (an air bubble curtain, two floating booms with a wheel) is a first key aspect. The intrinsic characteristics of the plastic and biota items (e.g., size, shape) as well as the number of items present have been identified as a second important set of key aspects. Finally, the hydraulic condition (flow velocity) of the waterway in which a mechanism is deployed is also incorporated. Based on the model with the highest technical reliability, simulations will be made to provide relevant scenarios and to evaluate the effect of the variables (e.g., flow velocity) on plastic and biota collection and removal.

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5.10.P-We469 Enhancing Environmental Sustainability through Industrial Symbiosis in Plastic Waste Recycling

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Plastics, primarily derived from fossil-based feedstocks, face significant end-of-life (EOL) challenges, with a large volume of plastic waste being diverted to linear options such as landfill, incinerators and the environment. European Green Deal's objective for circularity and climate neutrality aims at combining mechanical and chemical recycling, by employing circular economy principles of retaining resources at their highest possible value. This strategy creates a path towards a more sustainable manner for managing plastic waste. This study investigates the environmental benefits of integrating mechanical and chemical recycling methods within a circular economy framework. Two common plastics, low-density polyethylene (LDPE) and polypropylene (PP), were analyzed, using input-output data gathered from the literature, across various EOL scenarios. Life Cycle Assessment (LCA) using SimaPro was applied to analyze the environmental impacts of the two supply chains independently, from monomer production to EOL, across four scenarios: mechanical recycling with incineration or landfill, mechanical and chemical recycling, and the fourth being a replica of the third with the incorporation of industrial symbiosis between the two supply chains.

Largest environmental impacts from human health and ecosystem were witnessed in the scenario that combined mechanical recycling and incineration. Processing of the polymer accounted for the largest share in each process (for human health 34-46% for PP waste, 32-43% for LDPE waste, and for ecosystems 26-39% for PP waste and 37-51% for LDPE waste). Pyrolysis products led to avoided impacts of 14% for propylene from wPP and 9% for ethylene from wLDPE, due to the reduced virgin feedstock reliance. Industrial symbiosis achieved the lowest impacts in all the impact categories chosen, leveraging waste pyrolysis and material exchange to minimize resource extraction and environmental burdens. Integrating industrial symbiosis with mechanical and chemical recycling maximizes environmental benefits by reducing resource impacts and dependence on virgin materials, highlighting its potential for enhancing circularity in plastic waste management. This framework can guide policymakers and industries toward sustainable plastic waste management strategies.

5.10.P-We470 Using Life Cycle Assessment to Evaluate the Potential for Compostable Plastics

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Growing concern regarding the accumulation and management of traditional plastics waste has promoted interest in bio-based and biodegradable plastics due to their promising end-of-life i.e. biodegradation. However, to ascertain the sustainability of biodegradable plastic and enable consumers and producers to identify more sustainable methods of use, production, and disposal for such products, measurement of their environmental impacts is important. The application of comprehensive Life Cycle Assessments (LCA) studies are imperative to carefully evaluate sustainability of bio-based and biodegradable plastics. However, many life cycle assessments of these plastics do not consider the EOL phase due to a lack of consistent data, even though this stage can strongly affect the conclusions. Therefore, the goal of this study was to analyse published LCA studies on biodegradable plastics, focusing particularly on compostable plastic and the way end of life of the plastics are modelled i.e. composting. Gaps in current data are highlighted along with improvements needed to give an accurate environmental picture of compostable plastics. The results show that there is no general approach to model composting processes. There is a lack of data on timing of CO₂ emission, consequences of applying compost on soil and specific material degradation rate.

5.10.P-We471 Environmental and Economical Assessment of Enzymatic Depolymerization for Polyethylene Terephthalate (PET) Waste

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Traditional recycling of polyethylene terephthalate (PET) through mechanical processes has limitations. Specifically, low-purity PET waste is often diverted to incineration, which contributes to greenhouse gas emissions. Enzymatic depolymerization shows potential for treating low-quality PET by breaking it down into high-quality monomers. This study critically evaluates the environmental and economic feasibility of enzymatic depolymerization as a sustainable recycling method for low-purity PET waste. Accordingly, life cycle assessment (LCA) and life cycle cost analysis (LCCA) were applied to comparatively assess enzymatic depolymerization (at lab and pilot scale), mechanical recycling, and incineration. The comparison is based on a functional unit of 1 tonne of low-purity PET waste within the European Union in 2024. The Environmental Footprint (EF) 3.1 method within the EASETECH software quantifies environmental impacts across five specific scenarios, addressing climate change potential. Enzyme production was identified as the main environmental hotspot, with significant impacts from inorganic chemicals at pilot scale and organic chemicals in laboratory scenarios. Findings at pilot scale indicate that enzymatic depolymerization can reduce greenhouse gas emissions by over 2500 kg CO₂-equivalent per tonne compared to incineration, presenting a clear environmental advantage. The LCCA indicates that capital expenditures (CAPEX) are largely dominated by equipment installation and procurement costs, due to the need for advanced machinery. Operational expenditures (OPEX) are dominated by enzyme synthesis costs, especially for Isopropyl-β-D-thiogalactopyranoside (IPTG), which is essential in enzyme production. Additionally, the generation of valuable by-products, such as terephthalic acid (TPA) and ethylene glycol (EG), enhances both the environmental and economic viability of enzymatic depolymerization over traditional recycling methods. Despite scalability and data precision challenges, the findings suggest that enzymatic depolymerization offers a promising pathway toward circular economy goals. Optimizing chemical inputs and reducing costs are critical for advancing this approach to widespread application. This comprehensive analysis provides valuable insights into the potential for enzymatic PET recycling to drive sustainable waste management and significantly contribute to circular economy objectives.

5.10.P-We472 Processing of Wastewater Sludges From Plastic Recycling: An Lca Perspective

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The recycling of flexible low-density polyethylene (LDPE) foils often requires prewashing, which leads to the production of wastewater sludges with high plastic content. Using these sludges for alternative end-of-life applications rather than landfilling or incineration is desirable but poses the question of the efficiency and sustainability of such solutions. Generally, the most researched use of wastewater sludges is in the production of construction materials.

Three such production methods were developed and tested on wastewater sludges from a recycling facility in Central Bohemia, whose dedicated wastewater treatment line produces two types of high plastic content sludges with different compositions. These three applications are: a clarifier in brick production, a filler in concrete production, and an input for plastic roof tile production. This study evaluates the sustainability of each of those three newly developed applications using life cycle assessment (LCA). The environmental

impacts of these applications are assessed in the cradle-to-gate system boundaries using the EF 3.1 methodology. Each application is evaluated including possible benefits, e.g. resource savings compared to the original production method. A suitable mix of waste sludge management methods is presented that promises the most benefits and therefore the least environmental impact.

The LCA results show that for some of these applications, such as clarifier in brick production, the benefits are undeniable. For other applications, the benefits are heavily dependent on the energy consumption and choice of particular production methods. A thoughtful combination of these applications with consideration of their environmental impact could bring efficient utilization of sludges, thus saving of resources. Additionally, the perspective of environmental impact assessment highlights the potential for scale-up and opens the horizon for industrial application.

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5.10.P-We473 Environmental Impacts of a Circular Textiles Ecosystem in the UK with Automated Sorting and Fibre-to-Fibre Recycling

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Currently around 750,000 tonnes of non-reusable post-consumer textiles are generated in the UK every year, with just under 390,000 tonnes of this being disposed of directly through residual waste streams into landfill and incineration. At the same time, textile production is only increasing, with a reliance on virgin materials, since only 1% of textiles are currently recycled back into textiles. Balancing this out and closing the loop requires innovation in collection of post-consumer textiles, sorting and fibre-to-fibre recycling, but the environmental impacts of such a system change are unclear. This project looks at the impact of one potential solution, widespread automatic sorting facilities, feeding into scaled-up fibre-to-fibre recycling processes within a fully circular textiles ecosystem. Through collecting both primary and secondary data covering the full system, a Life Cycle Assessment (LCA) was conducted to understand the overall impacts and benefits, and identify hotspots in this that requires further innovation and development. Results suggest that overall, the system has great potential for providing environmental benefits, but there are several important considerations that will need to be taken into account on the transition to ensure these benefits can be realised. Firstly, recycled content targets for brands and retailers need to consider the virgin material production that will be required for blending with recycled fibres to achieve quality and durability. Secondly, there will need to be a diversity of solutions to balance out the hotspots in impacts for different technologies. For example, chemical recycling may have higher impacts, but more useful products, than mechanical or thermo-mechanical closed-loop recycling. Conclusions from this research have been distilled into a wider business case considering economic and social impacts as well as environmental. Findings from this work have not been confirmed, but will feed into planning for the development of a circular textiles system with investors, policymakers and businesses.

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5.10.P-We474 Advanced Analytical Strategies for Recovering Technology-Critical Elements from E-Waste: A Path Toward Circular Economy

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In our rapidly advancing technological world, the demand for technology-critical elements (TCEs) such as gallium, germanium, indium, niobium, rare earth elements, and tantalum has dramatically increased. Yet, without efficient recycling, these valuable materials become inaccessible after their life cycle, leading to resource inefficiency. This is particularly critical for electronic waste (e-waste), often referred to as an "urban mine" for its immense but underutilized resource potential. Accurately determining TCE concentrations in e-waste is crucial to unlocking its economic value and supporting sustainable recycling aligned with circular economy principles.

This study addresses the analytical challenges posed by the complex chemical matrices of e-waste materials, including printed circuit boards (PCBs), light-emitting diodes (LEDs), and lithium-ion batteries (LiBs). Using a combination of digestion-based inductively coupled plasma mass spectrometry (ICP-MS) and laser ablation ICP-MS (LA ICP-MS), we developed a robust methodology for precise elemental analysis. Optimized microwave-assisted acid digestion, with a mixture of 1 mL HCl, 3 mL HNO₃, and 1 mL HBF₄, achieved high accuracy and recovery. This method enabled the quantification of 58 elements, including valuable TCEs like Cu, Li, and Ni, with significant economic implications. For example, Cu in

PCBs was measured at 112,000 mg/kg (8.99/kg), and Li in LiBs at 21,000 mg/kg (8.34/kg), yielding total values of 15.80/kg for LiBs, 16.14/kg for PCBs, and 5.71/kg for LEDs.

However, the heterogeneity and complexity of e-waste requires alternative techniques. To complement the digestion results, LA ICP-MS was used, which allows almost direct analysis of solid samples. Combining the strengths of both methods, this dual approach increases reliability and precision, overcoming the limitations of digestion while also providing spatial resolution and ensuring validity of results.

The findings contribute to a deeper understanding of how advanced analytical techniques can support resource management practices. By bridging technical challenges and broader goals such as resource efficiency and sustainability, this study offers practical insights into unlocking the potential of urban mining while supporting the transition toward a circular economy.

5.10.P-We475 Environmental Cost-Benefit Analysis of Zero Waste Cosmetics

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More than 350,000,000 tons of plastic waste are produced every year, and a large part of them can end up mismanaged and eventually reaching water bodies. In these environments, plastics break down into micro- and nanoplastics, contaminating water that is recirculated for our daily uses and harming marine and river ecosystems. To address this issue, numerous plastic-free products have been developed in recent years. However, selecting the best options for production and purchase remains a challenge.

The outputs of Life Cycle Assessment (LCA) in terms of Environmental Footprint can be the answer to provide specific information about the magnitude of the different impact categories of the whole life cycle of different products. Moreover, whenever it is possible to turn these impacts into costs thanks to specific monetization factors it is also possible to include LCA results in a wider costs-benefits analysis of the products. When this happens, LCA plays a double role for decision making and reporting, providing essential information both in environmental and economic terms. This use of LCA can be helpful for a more effective comparison between different products that have the same function.

In this study, the comprehensive cost-benefit analysis (CBA) of solid shampoo balls will be presented as an example of double contribution of LCA. This product consists of a plastic-free alternative to liquid shampoo that eliminates the need for plastic packaging, and which features a less polluting chemical formulation. As such, it will contribute to the valuable insights into plastic-free alternative products for informed decision-making by different stakeholders. As an example, CBA can provide a foundation for (i) manufacturers and producing companies that need to understand which options are the most feasible, both practically and economically, (ii) investors that need to understand which technologies will create less damage to the environment, in order to ensure a higher reputation and a lower investment risk, and (iii) customers in order to understand which products of daily use may be the least impacting for their own health and the environment.

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5.10.P-We476 Comparing Past and Future Organic Waste Management Approaches for Montreal (Canada) Using Life Cycle Assessment

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Canada is moving towards a low-carbon and circular economy by increasing diversion of organic residues to composting and anaerobic digestion. Diverting organic residues from landfills can reduce greenhouse gas emissions associated with the biological decomposition of organic matter. However, the carbon footprint and other environmental impacts of organic waste management systems in Canada are currently poorly understood. The goal of this research is to quantify the environmental impacts of the present and future organic waste management scenarios for Montreal using Life Cycle Assessment (LCA). In the past scenario, organic waste was transported away from the urban center and treated by open windrow composting. In the future scenario, organic waste will be managed by a combination of closed tunnel composting and anaerobic digestion, embedded within the metropolitan area. The Solid Waste Optimization Life Cycle Framework in Python (SwolfPy), an open-source waste LCA software using Brightway2, was used to perform the life cycle inventory modeling and impact assessment calculations. Our analysis reveals distinct tradeoffs between different treatment regimes. For instance, reductions in emissions from transporting waste outside the city can be counteracted by those from energy inputs to

maintain closed tunnels and run the anaerobic digestion process. By identifying these and other tradeoffs, this analysis addresses longstanding knowledge gaps in the treatment of organic waste in Canada and supports informed, sustainable management of urban waste streams in the country and beyond.

5.10.P-We477 3D Printed Artificial Reefs: LCA and LCC Analysis to Compare Traditional Cementitious Mortars and Innovative Mixtures Made From Bivalve Mollusk Shells

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Restoring marine biodiversity is a crucial challenge to ensure healthy ecosystems capable of counteracting the loss of the global ecological network. Integrating circular economy practices into habitat recovery helps the reduction of the environmental impact, the optimization of resource use, and the implementation of systemic sustainability. This study presents a comparative analysis of the environmental and economic performance of 3D printed artificial reefs, made either from traditional cementitious materials or from an innovative composite derived from the reuse of mussel shells.

The reefs, designed for the repopulation of the native European flat oyster *Ostrea edulis*, aim to improve local marine biodiversity and regenerate compromised marine habitats. The circular economy approach utilizes waste shells from mussel and oyster farming, transforming them into a valuable resource to replace natural aggregates in construction materials. The innovative material consists of a high percentage of shells, which are over 90% composed of calcium carbonate, making them a sustainable alternative to traditional construction materials.

The Life Cycle Assessment (LCA) will evaluate the environmental impacts of the materials, comparing conventional cement mortar with the shell-based innovative material. A cradle-to-gate approach will be adopted, analyzing the impacts related to raw material extraction, processing, transport, and use. The European footprint method will be applied, considering its environmental impact categories. In parallel, the Life Cycle Costing (LCC) analysis will provide a comprehensive economic perspective to assess the foundations needed to establish a value chain for the valorization of a bio-material.

The project aims to generate concrete data to promote the use of sustainable materials in marine regeneration. The planned analyses will help identify optimal scenarios for marine ecosystem restoration, balancing costs and environmental benefits, and highlighting how the principles of sustainability and circularity can be integrated into conservation and technological innovation projects.

5.10.P-We478 Life Cycle Assessment of UK Household Leftovers: Implications for Storage and Disposal Choices

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Household food loss and waste (FLW) contributes 18 million tonnes of CO₂-eq greenhouse gases (GHG) emissions annually in the UK. Almost 30% of them are well-cooked but not consumed, which is commonly referred to as leftover. Only less than one-third of leftovers is disposed through low-emission options such as anaerobic digestion (AD) or home composting. Consequently, it is necessary to store and eat leftover, which extending shelf-life of leftovers and minimising waste amount. Additionally, it helps to cut down on the need of prepare new meals, which in turn reduces the upstream production and associated emissions.

In this study, the life cycle assessment (LCA) method is conducted to evaluate the environmental impacts of household leftovers in the UK, comparing between storing and re-consuming 1 kg of leftovers, versus disposing of them as biowaste through AD and preparing a new meal. Additionally, different storage methods are assessed to identify the most suitable one for saving leftover. Furthermore, sensitivity analyses are applied to account for variations in national electricity grids and cooking methods, to assess the extent to which these factors influence the decision of preserving leftovers.

Results show that there are 3.95 kg CO₂-eq GHG emissions during the AD process, which is 2.23% reduction compared to cooking a new meal. This means the current UK choice of AD as the main biowaste disposal method is acceptable. However, the offsets from disposal process are minimal, with the main input are from upstream processes of leftover, such as production. Therefore, storing and re-consuming leftover results in significantly lower emissions compared to new meal. For instance, storing leftovers in glass containers produces 0.046 kg CO₂-eq emissions, accounting to a 98.9% reduction. Sensitivity analysis reveals that, when considering different reheating methods, reheating on gas stoves offers averagely 32% more benefits than using microwaves. Furthermore, due to the electricity usage of refrigerator and reheating, electricity grids also affect the emissions, vary from a 70% reduction to a 200%

increase. Generally speaking, despite these variations, the emission reductions achieved by storing and re-consuming leftovers consistently exceed 95% compared to preparing a new meal, indicating that the effective strategy to mitigate environmental impact of leftover is to store and re-use them, rather than relying on other disposal methods.

5.10.P-We479 Life Cycle Assessment (LCA): Conventional Thermal Energy Storage (TES) System Versus Alternative Steel Slag-Based System for Concentrating Solar Power Plants (CSP)

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Thermal Energy Storage (TES) plays a crucial role in advancing decarbonisation. Its integration into Concentrated Solar Power (CSP) plants can significantly enhance efficiency and support renewable power generation. The primary commercial TES material, solar salt (SS), presents technical challenges and has the highest environmental impact in TES systems. An innovative alternative is Electric Arc Furnace Steel Slag (EAFSS), a steel industry waste that can be repurposed as TES material. However, the environmental sustainability of EAFSS for TES applications has not been comprehensively studied. This research quantified the environmental impact of a thermocline TES system using EAFSS as a filler material, compared with a conventional SS system. A Life Cycle Assessment (LCA) examined EAFSS environmental burdens employing mass and economic allocation methods. The LCA scope encompassed a "cradle-to-gate" perimeter, which covers the manufacturing stage of a CSP tower plant. The results showed that EAFSS provided the same storage capacity as the conventional system while reducing 9% the TES' material intensity. At the TES system level, the ReCiPe method indicated impact reductions of 21% using mass allocation and 53% with economic allocation. When using mass allocation, the global warming emissions were 49% higher (127 kg CO₂eq/kWh) than in the conventional TES system (85 kg CO₂eq/kWh), which may benefit steelmaking industries by attributing a large share of their emissions to CSP plants. In contrast, economic allocation yielded lower emissions (58 kg CO₂eq/kWh), providing more credit to recycling EAFSS in TES systems. These findings underscore the potential of EAFSS to enhance CSP sustainability while valorising waste/by-products to generate greener electricity.

5.10.P-We480 Safe and Sustainable Recycling of Solar Panels: A Scenario Study

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To assess and stimulate safe and sustainable recycling the National Institute of Public Health and the Environment (RIVM) developed a screening methodology to assess the circularity, environmental impact and safety of recycling of waste flows. This method was applied on the case of recycling of End-of-Life solar panels. It is expected that the first generation of solar panels will start to be taken out of use in large numbers in five years time. Various technologies to recycle solar panels are being developed.

The aim of this study was to investigate which recycling options appear to be feasible, how environmentally friendly they are and to learn how the screening methodology can help to make a comparison. We compared four future scenarios to the current situation (the baseline), in which no raw materials from End-of-Life solar panels are recovered, but are shredded and processed for a low-grade applications like road foundation. Three screening modules were applied for quantifying the circularity, environmental impact and the safety due to the presence or emission of substances of concern (SoC). Our analysis shows that all future options are more circular and have a lower environmental impact than the baseline. One scenario scores best on climate change (CO₂-eq.), but all are much lower than the baseline. One of reasons is that it costs more energy to process new raw materials into solar panels than to use recycled raw materials. The option in which glass is recycled into new solar panel glass and the silicon can be recycled to high grade silicon, has also the highest value for the circularity indicator 'efficiency'. In the recycling process, attention should be paid to hazardous substances in solar panels: lead, antimony and per- and polyfluoroalkyl substances (PFAS). Lead is contained in soldering materials and antimony is added to make the glass brighter. The backsheet contains fluoropolymers, as a result of which PFAS can be released when they are incinerated. This can lead to human and environmental exposure, depending on how substances are released.

Based on this study we advised the national government to stimulate specific technological developments that enable high-grade recycling of solar panels. We also recommend to stimulate design for recycling. For example developing other encapsulant materials between the glass and the backsheet enables easier dismantling. It is also important to minimise the use of hazardous substances lead and PFAS.

5.10.P-We481 Bottom-Up, Dynamic Probabilistic Material Flow Analysis of Japanese Plastic Flows as an Initial Step to Understand the Chemical Additives Flows and Stocks

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The global production of secondary plastics has increased more than tenfold in the past two decades, creating a strong backflow from end-of-life (EoL) products to manufacturing. Secondary materials can substitute pristine resins in manufacturing, and therefore mitigate many of the related environmental impacts. However, the associated risks of secondary materials, particularly from the additives that persist through recycling processes, are not yet fully understood. Tracking the flows of chemicals in secondary materials requires high-resolution data on plastic flows. As a first step of achieving so, this study presents a dynamic probabilistic material flow analysis of plastic flows, with high granularity at the EoL stage. Japan was selected as a case study, due to the availability of comprehensive data. We compiled the production, trade, and consumption data for eight major types of commercial polymers for the period from 1950 to 2023. The amounts were allocated to 48 product categories following a bottom-up approach based on the annual sales data and the polymer composition of these products. Using the consumption data and lifetime distributions, we estimated waste generation for individual product categories. Twenty-three collection pathways were differentiated across general waste collection, industrial waste collection and sorted collection for specific product categories that are regulated under existing waste laws and/or managed by various industrial associations. The inputs and outputs of mechanical recycling were modeled accordingly. Our results show a significant increase in recycling activity following the establishment of respective recycling laws. However, the absolute quantities of secondary plastics remain relatively low compared to those that were sent to energy recovery and landfill. Secondary materials primarily originate from containers and packaging, electronics, and motor vehicles. These findings provide initial insights into the types of secondary polymers and chemicals likely present therein, forming a basis for future quantification of the flows of chemical additives and their environmental and human health impacts.

5.11.A Food for Thought: How Do We Move Towards a Safe and Sustainable Global Food System?

5.11.A.T-01 Towards Sustainable Agri-Food Systems: Environmental Benefits of Climate-Smart Agriculture in Norway

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Climate-smart agriculture (CSA) practices aim at contrasting land degradation and enhancing climate resilience to ensure safe and sustainable food systems. The environmental benefits of CSA often go undetected and appear negative in life cycle assessment (LCA), which typically focuses on product-based outputs, overlooking ecosystem services and resilience. We explore how CSA can be better represented in LCA of agricultural practices by investigating barley production on cropland at risk of soil erosion in Norway under three management scenarios: conventional agriculture, integration of winter cover crops, and establishment of trees as buffer zones. The assessed environmental impacts combine ecosystem services (reduction of soil erosion, habitat quality, carbon sequestration) with traditional life-cycle impact categories (climate change, freshwater and marine eutrophication, human toxicity, terrestrial acidification and freshwater ecotoxicity). Relative to the conventional barley production system, buffer zones improve all environmental impacts (26% on average) and cover crops all but terrestrial acidification (10% on average), when using a land-based functional unit. When a product-based functional unit is used, buffer zones still perform better than conventional farming (but relative benefits are reduced to 21% on average) while cover crops show higher impacts in five impact categories (and the average benefit is decreased to 8%). Both approaches always deliver higher ecosystem services under both types of functional units, despite the lower crop yields. Specifically, buffer zones and cover crops increase habitat quality, terrestrial carbon storage and reduce nutrient and soil-particle runoff, thereby supporting long-term yields and improving the circularity of this food system. Reduced short-term yields of the main crops remain a barrier to CSA adoption. Identifying the environmental advantages of these practices can provide evidence to support the transition to more safe and sustainable agricultural systems.

5.11.A.T-02 Using Large-Scale Farm Surveys to Identify Productivity and Sustainability Solutions

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India is one of the world's largest crop producers, with its agricultural sector being responsible for 18% of its total national emissions. Life Cycle Assessments (LCAs) are crucial for attributing environmental impacts to specific products and farms and identifying areas where impacts can be reduced. However,

there has been little work so far on converting LCA results into sustainability improvement advice for farmers. Farmers need reliable, up-to-date information on mitigation measures they can adopt, and how such measures may affect other important areas such as crop yield. This research focuses on converting large-scale agricultural survey data into LCA results, and then using these results to create mitigation solutions for farmers. Specifically, we use farm survey data from the International Maize and Wheat Improvement Centre (CIMMYT), covering over 30,000 crop production cycles, 22 unique crops, and site locations spread across India. We format the data and then upload it onto HESTIA, a platform that archives and makes available food production and impact data in a consistent format. HESTIA has over 500 models available which automatically gap-fill the data (for example by calculating diesel inputs based on the operating hours of machinery) and calculate emissions, resource uses, and characterised indicators for every crop cycle. We generate univariate distributions of production practice and environmental impact data, which enables us to identify plausible ranges of inputs, crop yields, and emissions. We also generate multivariate distributions, allowing us to understand how one variable (e.g., fertiliser or soil type) varies with another (e.g., crop yield), which then allows us to identify the main sources and drivers of impacts. Using these results, we generate profiles of what the lowest environmental impact farms look like in each region based on current practices. Finally, we go beyond this to use sensitivity analysis to include new production practices (e.g., zero tillage, agroforestry, or zero crop residue burning) to explore how environmental impacts could be further reduced. Overall, this allows us to create a list of possible mitigation options for any given farm in India. Our approach is scalable and extensible to other farming systems, and we demonstrate this by applying our methods to data from another large farm survey (~40,000 rice farms in India, Nepal, and Bangladesh), that is a part of the Landscape Crop Assessment Survey (LCAS).

5.11.A.T-03 Can 'Superfoods' Bridge Nutritional Gaps and Promote Environmental Sustainability in Spain? A Proposal for a Context-Specific Nutritional Model in Life Cycle Assessment

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The overriding connection between climate interactions and nutritional outcomes of food systems is at the forefront of research, especially when it comes to assessing alternative food products. To address this issue, significant progress is being made in the field of nutritional life cycle assessment (LCA), with the development of novel methodologies and specific approaches garnering increasing attention within the LCA community. In this context, the objective of this investigation is to design a nutritional index, namely nutrient profiling (NP) model, adapted to the Spanish context for use in nutritionally-factored environmental life cycle assessments of superfoods.

The development of the NP model is based on iterative decision-making, where design characteristics such as the objective, the type of model, the criteria or the nutrients included have to be decided. The nutrient rich family of models is taken as basis for our system, and the specification is guided by the contextualization. Qualifying and disqualifying nutrients are selected based on the critical nutritional shortfalls of the Spanish population and associated health outcomes, and weighting factors are included motivated by the capacity of superfoods to meet recommendations.

The integration of the features gives rise to the Spanish Nutrient Rich (super)Food 9.2 (sNRF9.2) model, which proves its validity by aligning with the Spanish Public Health Strategy and other NP models. The application of the model as functional unit in LCA studies of super and conventional foods shows its usefulness in conveying integrated information efficiently. Trends reveal a conflicting situation for a wide range of products, including fruits, fish, cereals, and vegetables, where greenhouse gas emissions are low, but so is the nutritional quality of the food. In contrast, nuts, seeds and legumes represent the best alternative for its good nutritional-environmental balance. Among all products tested, two superfoods, namely, oat and carob seeds, are located at the top ten of the ranking, evidencing the potential of alternative nutrient sources. However, despite the model's applicability and versatility, it is important to acknowledge the need for periodic updates to accurately reflect the environmental and nutritional landscape in Spain.

5.11.A.T-04 Safety of Circular Food Systems: Understanding the Fate of Chemicals

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The re-use of residual streams in the food system becomes more important, especially to close agricultural production loops. Residual streams contain nutrients, which can be re-introduced in the food system. However, besides nutrients, chemical or microbial hazards may also be present in residual streams which then can be re-introduced or accumulate in the circular food system.

One potential useful residual stream to use as a nutrient source to close agri-food production loops is

sludge. Sludge of wastewater of domestic origin (STP) as well as sludge of wastewater of the agro-food industry (FTP) were collected in The Netherlands, and has been characterized for their presence of chemical, microbial, and physical hazards. The sludge can again be used as a nutrient source in multiple circular food systems. In this study, the application of the sludge samples as a nutrient source for edible insect rearing (black soldier fly larvae; *Hermetia illucens*) was evaluated. The fate of multiple chemical hazards was evaluated. These included heavy metals and trace elements, of which especially cadmium accumulated in the black soldier fly larvae, with bioaccumulation factors all exceeding 1. Also, the presence and fate of short chained and some longer chained per- and polyfluoroalkyl substances (PFASs) was evaluated. Highest concentrations in the sludge samples were determined for trifluoroacetic acid (TFA), which were detected in only some of the corresponding insect larvae samples, in which no bioaccumulation was reported (bioaccumulation factor <1). Longer chained PFASs, such as PFOA, PFOS, PFHxS and PFNA, were evaluated to bioaccumulate in the black soldier fly larvae in most of the exposure conditions. Bioaccumulation factors were generally highest for PFOS. This corresponded to findings of another study in which black soldier fly larvae were exposed to these four PFASs single and in mixture, due to spiking of the start substrate (van Dongen et al., in preparation). This also resulted in bioaccumulation of all these four PFASs, with highest bioaccumulation factors for PFOS. Altogether, this study provides better insights into the fate of contaminants in a circular food production system, based on insect rearing for the production of alternative proteins.

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5.11.P-Th348 Assessing Impacts on Biodiversity on an Aquaculture Portfolio

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The increasing global demand for sustainable practices is pushing companies to better assess and disclose their environmental impacts, particularly on biodiversity. In Europe, the Corporate Sustainability Reporting Directive (CSRD) reinforces this need by requiring businesses to report on their biodiversity impacts. In the seafood sector, aquaculture plays a central role by surpassing production from capture fisheries and contributing nearly 60% of seafood produced globally. However, reliable biodiversity data remain limited, and existing third-party sustainability certifications, such as those from the Aquaculture Stewardship Council, cover less than 4% of global aquaculture production.

To support companies falling under the scope of this directive, we developed a qualitative, risk-based method to assess biodiversity impacts across aquaculture portfolios. The approach considers IPBES's (Intergovernmental Platform for Biodiversity and Ecosystem Services) five drivers of biodiversity loss and focuses on two key stages of the value chain: feed production and farming practices. In total, eight indicators are qualitatively evaluated to assess risks from "cradle to farm gate."

We tested this methodology on two key aquaculture species: Atlantic salmon (*Salmo salar*) and Whiteleg shrimp (*Litopenaeus vannamei*), spanning diverse practices including ASC certified operations and more traditional (non-certified) and countries of origin. Results revealed notable differences in biodiversity impacts between sustainable and conventional production methods. Certified products consistently demonstrated lower risk levels, providing evidence of best practices due to the strict requirements, burden for evidence and supply chain assurance that are part of the ASC certification program. The model emphasizes key impact areas (e.g. deforestation, pollution, fishing pressure) and enables companies to pinpoint risks, prioritize mitigation measures, and guide strategic decision-making. Our risk-based approach fills a critical gap in biodiversity data and provides a scalable framework for aquaculture portfolio assessment. It supports businesses in gradually improving their biodiversity knowledge and aligning with CSRD requirements.

Further pilot studies are underway to refine the methodology, including the consideration of indicator weighting and cumulative scoring mechanisms. These advancements aim to drive meaningful improvements toward more sustainable aquaculture practices.

5.11.P-Th353 A Sustainable Transportation System Will Require Sustainable Dietary Patterns and Food Systems: Focus On Cycling Life-Cycle Impacts Including Additional Calories Intakes and Regional Diet Evolutions

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Increasing active mobility cycling, walking seems a promising lever to mitigate impacts to climate change, but switching to active modes requires to produce more food to supply additional calorie intakes (ACI). Accounting for more than 1/3 of the global land distance traveled by passengers, cycling also requires substantial extra calories, making its environmental impacts intrinsically linked to food system sustainability. This study aims to explore (a) the life cycle impacts of different cycling practices by region and type of bike including ACIs, and (b) the share of cycling environmental impacts in prospective mobility scenarios under different archetypical regional diet evolutions.

The method is based on : (1) modeling scenarios of global future transportation demands for land passenger travel, (2) conducting the LCA of transportation modes, (3) assessing the environmental impacts of the scenarios. (1) is an extrapolation of the International Transportation Forum scenarios, where technologies by mobility mode are completed using other sources. In (2), we develop regional archetypes of conventional modes to conduct prospective LCAs using premise. We gather existing and develop new life cycle inventories of bikes (electric/mechanical (e-/m-), standard/sportive/cargo, aluminium/bamboo/carbon), model calories consumed depending on regional cycling practices (speed/intensity) using the Metabolic Equivalent model, as well as different dietary patterns (vegetarian, keto, 6 average regional diets), to calculate the life cycle impacts of different cycling practices and transportation scenarios using IMPACT World+ (3).

Cycling environmental impacts change highly when considering ACI. The carbon footprint of cycling in Africa goes from 11 to 26 gCO₂e/km, while at the other extreme, it jumps to 95 gCO₂e/km in Europe. Under a keto diet, cycling with a m-bike emits 135 gCO₂e/km, what is like using an e-car with a green electricity. Under highly emitting diets, e-bikes perform better, but e-biking or m-biking emits similarly under a vegetarian diet (35 g). Under stable diets and food systems but high decarbonization ambitions in the transportation sector, cycling would reach by mid-century 1/6 of the contributions of the global land transportation to CC and water scarcity, and 1/3 to its land occupation and marine eutrophication, mostly due to ACIs. Becoming all vegetarians mitigates some impacts but may make the contribution to water scarcity explode.

5.11.P-Th363 Chemicals of Concern in Food Packaging: A Matter of Sustainability

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In a globalized system, food is increasingly processed and transported over long distances. This is enabled by highly functional food packaging. However, food packaging is also a driver of long-term damage to the environment and human health. From the sourcing of raw materials over production to end-of-life, food packaging has multiple impacts on the use of resources, environmental pollution, and exposure to chemicals. Therefore, decision makers in the food sector need transparent and science-based information to choose safe and sustainable food packaging for each type of food.

The UP Scorecard was developed for this purpose and is built on a scoring system, including the six impact areas climate change, water use, plastic pollution, sustainable sourcing, recoverability, and chemicals of concern. While other packaging assessment tools are available, they often focus on a single impact area, such as climate change, but usually omit the chemical safety of a material.

To assess chemicals of concern within the UP Scorecard, we applied a hazard scoring system to over 14,000 known food contact chemicals (FCCs), based on the methodology described in the PlastChem report (DOI: 10.5281/zenodo.10701706). We focused on the human health hazards only, as there is direct oral human exposure from the many chemicals that migrate from packaging into foodstuffs. In total, we found 4680 FCCs with hazard scores indicating different levels of concern for human health. 419, 121, and 406 out of these FCCs have the highest hazard score for carcinogenicity, mutagenicity and reproductive toxicity, respectively, whereas 45 FCCs are confirmed endocrine disrupting chemicals, and 1330 FCCs have specific organ toxicity.

To facilitate access to this information for decision-makers, such as procurement professionals, we grouped the chemicals of concern based on their structural properties but also integrated filtering options regarding the use and presence of FCCs in food contact materials. This will help to identify, screen and avoid the most hazardous chemicals for each food packaging material and support development of safe and sustainable food packaging solutions.

5.11.B Food for Thought: How Do We Move Towards a Safe and Sustainable Global Food System?

5.11.B.T-01 Contribution of House Gardening Towards Sustainable Food Systems in the EU

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The food system stands at the intersection of many environmental crises, and it needs to transform both to mitigate environmental impacts, as well as to adapt to the changes in Earth System functioning. House gardening can be an important piece of the puzzle of a sustainable food system, yet there are currently lacking larger scale quantitative assessments of environmental consequences of a widespread adoption of gardening. In this study we aim to fill this gap and evaluate what role house gardening can play in the EU. We map the area potentially usable for house gardening in the EU27 states based on OpenStreetMap data. We evaluate the life cycle environmental impacts of vegetable cultivation in gardens and quantify the potential benefits of substituting conventional vegetable production. We compare two scenarios evaluating both direct one-to-one substitution and indirect substitution through market shifts evaluated using the agro-economic model CAPRI. The variability of underlying parameters and gardening practices is captured with a Monte Carlo simulation. The model-mean garden production, assuming 50% utilization of the potential area, is over 200 Mtons of vegetables, a value larger than the EU production and import in 2020 according to FAOSTAT. Based on the results, house gardening could lead to significant benefits across all impact categories. Direct substitution of conventional production could lead to avoidance of 25 Mtons CO₂ eq, with the potential benefits of house gardening for climate reduced to 5 Mtons CO₂ eq when market consequences are modeled. This study provides the first quantitative estimate of the potential of house gardening for sustainable food system transition in the EU. With the large variability of potential crops and gardening practices, and with the data and knowledge limitations, these values have the character of only rough estimates. Still, we show that the potential role of house gardening in a sustainable food system could be significant, especially considering other, less tangible benefits of gardening.

5.11.B.T-02 Rising Global Species Loss Embodied in Oil Crop Supply Chains

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Oil crop cultivation has emerged as a major driver of global biodiversity loss through demand-driven land use expansion. We quantified the global species loss embodied in oil crop supply chains from 1995 to 2020 by combining spatially explicit biodiversity impact assessments with a hybrid multiregional input-output model, enhanced supply chain mapping, and structural decomposition analysis. Our findings reveal that in 2020, the biodiversity impact from oil crop cultivation land use reached 0.016 global PDF (Potentially Disappeared Fraction), indicating that approximately 1.6% of species would potentially face complete extinction if current land use patterns for oil crop cultivation persisted. Tropical regions accounted for 77% of biodiversity impact from oil crop cultivation, predominantly due to the production of oil palm, coconut, and soybean. We observed significant outsourcing of biodiversity impacts, with increased imports to China, the European Union, and North America driving species loss in producer countries such as Indonesia and Brazil. Global biodiversity impact from oil crop cultivation increased by approximately 83% from 1995 to 2020. Over 90% of biodiversity impact growth from food end-uses stemmed from increased vegetable oil and animal product demand in the Global South, while the European Union dominated both total impact and impact growth in non-food end-uses. Structural decomposition analysis identified rising per capita consumption as the primary driver of increasing biodiversity impacts due to oil crop cultivation. To reduce biodiversity loss, non-food and feed uses of oil crops should be minimized and production in biodiversity hotspots avoided. Our findings underscore the urgent need for policies targeting sustainable production and consumption across global oil crop supply chains and provide a basis informing such actions.

5.11.B.T-03 Pathways to a More Sustainable Swiss Food System: Holistic Assessment Reveals Synergies and Trade-Offs

Thomas Nemecek, *Utkur Djanibekov, Albert Von Ow, Alba Reguant Closa, Vasco Diogo, Melanie Douziech and Gabriele Mack, Agroscope, Switzerland*

The Swiss Sustainable Food Systems (SWISSfoodSys) model allows the evaluation of various future scenarios for the development of the Swiss food system. The model has been completely revised and substantially extended by including economic indicators such as added value, costs and agricultural income, labour requirements, as well as nutritional value and health impacts of different diets. In this contribution, we used the model to assess future dietary patterns and food system optimisation strategies from a holistic perspective.

The SWISSfoodSys model uses a linear programming approach to simulate the development from 2019 to 2050. It includes 43 crop activities, 41 livestock categories, and processed 122 products, imports and

exports. The model integrates the Nutrient Rich Food Index and the Health Nutritional Index to assess the health impact of various diets. For Swiss production we generated Life Cycle Inventories (LCIs) with the Swiss Agricultural Life Cycle Assessment (SALCA) method. For imported goods, we calculated mixes of different countries of origin from available LCI databases, including the transports to Switzerland. SALCA LCIA has been used for the impact assessment.

We simulated a range of dietary scenarios (flexitarian, vegetarian, mix of different dietary patterns) and food systems optimisation scenarios (with different targets). All scenarios account for population growth over time. All dietary scenarios led to significant reduction in livestock production and an increase of domestic food production, thereby improving self-sufficiency. While following dietary recommendations resulted in a reduction of greenhouse gas (GHG) emissions, this was insufficient to meet climate targets. Reduction of food waste led to substantially lower environmental burdens in all scenarios. Additional system optimisation and dietary changes are needed to achieve the climate targets. First results indicate synergies and trade-offs between the different indicators, such as trade-offs between GHG emissions and agricultural income. Achieving a significant reduction in GHG emissions would require reduced livestock production and lower consumption of meat and milk, which would also decrease agricultural income. The SWISSfoodSys model allows to evaluate pathways to a more sustainable food system. By balancing different societal needs, good compromises between the different dimensions can be identified, which increases the chances of their implementation.

5.11.B.T-04 Biodiversity Impacts of Recent Land-Use Change Driven by Increases in Agri-Food Imports

Veronika Schlosser¹, Stephan Pfister², Stefanie Hellweg² and Livia Cabernard¹, (1)Technical University Munich, Germany, (2)ETH Zurich, Switzerland

Land-use change such as the conversion of natural habitat to agricultural land has been a major driver of global biodiversity loss, prompting efforts at biodiversity restoration. However, restoration measures in certain areas can shift the detrimental biodiversity impacts elsewhere through the outsourcing of agri-food supply chains to biodiverse regions. This study examines the link between biodiversity impacts from land-use change and shifts in global supply chains from 1995 to 2022. We combine spatially-resolved land-use change data from 1995 to 2022 with ecoregion-specific global species loss factors from UNEP-SETAC to assess both increases and decreases in biodiversity impacts from land-use change. We integrate this regionalized impact assessment into Resolved EXIOBASE34,5 (REX3), a highly-resolved MRIO database (189 countries x 163 sectors), and introduce a marginal allocation to study the link between shifts in global supply chains and recent biodiversity impacts from land-use change. Our study reveals that over 90% of global biodiversity impacts from land-use change are tied to international trade of agri-food products. Nearly 80% of recent global land-use change impacts were linked to agri-food exports from Latin America, Africa, and Southeast Asia-Pacific (excluding China). Conversely, increased imports to China, the United States, Europe, and the Middle East accounted for nearly 60% of global land-use change impacts, despite these regions experiencing decreased domestic impacts through restoration. This suggests that declining biodiversity impacts in temperate and arid regions are partly achieved by outsourcing agri-food supply chains to tropical biodiversity hotspots. As a result, global species extinction rates have accelerated, with a 1.4% potential species loss since 1995 exceeding the planetary boundary by about fifty times. This highlights the urgent need for policies incentivizing habitat protection and sustainable sourcing in agri-food supply chains.

5.11.P Food for Thought: How Do We Move Towards a Safe and Sustainable Global Food System?

5.11.P-Th346 Rapid Evidence Assessments to Increase Transparency of the Environmental Profile of Agricultural Practices to Inform Sustainability Improvements and Environmental Protection Goals

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Background: Agricultural systems are complex, with many interrelated components. Changing one practice to manage an environmental risk or hazard can inadvertently impact other risks and even result in more harmful environmental outcomes. A holistic approach that considers the overall environmental impact of the various management tools and practices will be required to steer agriculture towards more sustainable outcomes.

Challenge: Consequently, environmental risk assessments and their specific protection goals must realistically account for the impacts and adverse effects of diverse practices and seek to minimize trade-offs. The presented comprehensive approach building on rapid evidence assessments (REA) aims at supporting decision-making by revealing both advantages and limitations, and facilitating the exploration of synergies and trade-offs in agricultural practices.

Methods: A bottom-up approach was employed to rapidly review scientific publications on the environmental implications of common cropping tools and practices, such as inversion tillage, across twelve impact categories related to soil, water, climate, and biodiversity. The second phase builds on the REA results and focused on agricultural systems, analyzing the costs and benefits of various management options while considering soil health, climate change, water quality, biodiversity, and land use efficiency. **Results:** The rapid evidence assessment highlighted key environmental implications of various agronomic tools, making it easier to identify opportunities and trade-offs. When considering an overarching agricultural practice like conservation agriculture numerous environmental benefits but also limitations in temperate climates regarding productivity and land use efficiency were found.

Conclusions: The presented methodology was able to provide a more holistic view of the environmental impacts of various agronomic tools, including inversion tillage, crop rotation, and cover crops. By enhancing the understanding of the consequences of changing agronomic practices, this approach can support the development of outcome-based business models and regulatory approaches that incorporate biodiversity and ecosystem services, guiding decision-making and minimizing potential trade-offs in agricultural production.

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5.11.P-Th347 Circular Nutrient Management and Triple Planetary Crisis

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Nutrient chemicals are at the heart of the triple planetary crisis due to their role in pollution, biodiversity and climate change. Nutrients from chemical/industrial/mineral fertilizers and anthropogenic wastes are altering the biogeochemical cycles of N/P/K/S beyond planetary boundaries. As the ongoing international nitrogen and phosphorus assessments quantify the impacts of nutrient pollution, agriculture and waste management are in the spotlight. Intensive agriculture broke the natural circularity of nutrient linkages between crops and livestock, which derived nutrients from each other's wastes. Modern cropping practices in most countries rely excessively on fertilizers for nutrients, while wasting the same nutrients produced naturally by humans and livestock, causing nutrient/waste pollution from crops, livestock and humans. Globally, low fertilizer use efficiency or crops and poor management of livestock manure/urine and municipal wastes together account for most of the anthropogenic ammonia and nitrous oxide emissions to air pollution and nitrates/phosphates/sulfates to water pollution. Nutrient recycling from livestock manure/urine as well as domestic solid and liquid wastes into crop production can minimize fertilizer demand and save nutrient/waste pollution, human health, climate change, biodiversity and ecosystem services. While technologies and best practices are increasingly becoming available, policy frameworks are not yet conducive to fully recycling wasted nutrients and to use fertilizers only as top-up inputs when needed. Recently, UNEP global partnerships on nutrient management (GPNM) and wastewater (GWWI) and the UNEP resolutions on sustainable nitrogen management (UNEA 4/14 and 5/2) generated some intergovernmental attention. Academic, industrial and civil society attention is critical to amplify these initiatives and build up the momentum for sustainable nutrient management. This must begin with the restoration of legume-based multi-cropping and intercropping with other crops, as well as integration of livestock into every possible cropping system. Biological nitrogen fixation by legumes alone can reduce N-fertilizer demand by up to a third, while the rest can be met by recycling nutrients from manure/urine/compost/wastewater with similar crop yields. Further, crop nutrient demands can be reduced by ongoing crop improvement efforts for nutrient use efficiency through selection/breeding. Global/regional/sectoral examples will be shared.

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5.11.P-Th348 Assessing Impacts on Biodiversity on an Aquaculture Portfolio

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The increasing global demand for sustainable practices is pushing companies to better assess and disclose their environmental impacts, particularly on biodiversity. In Europe, the Corporate Sustainability

Reporting Directive (CSRD) reinforces this need by requiring businesses to report on their biodiversity impacts. In the seafood sector, aquaculture plays a central role by surpassing production from capture fisheries and contributing nearly 60% of seafood produced globally. However, reliable biodiversity data remain limited, and existing third-party sustainability certifications, such as those from the Aquaculture Stewardship Council, cover less than 4% of global aquaculture production.

To support companies falling under the scope of this directive, we developed a qualitative, risk-based method to assess biodiversity impacts across aquaculture portfolios. The approach considers IPBES's (Intergovernmental Platform for Biodiversity and Ecosystem Services) five drivers of biodiversity loss and focuses on two key stages of the value chain: feed production and farming practices. In total, eight indicators are qualitatively evaluated to assess risks from "cradle to farm gate."

We tested this methodology on two key aquaculture species: Atlantic salmon (*Salmo salar*) and Whiteleg shrimp (*Litopenaeus vannamei*), spanning diverse practices including ASC certified operations and more traditional (non-certified) and countries of origin. Results revealed notable differences in biodiversity impacts between sustainable and conventional production methods. Certified products consistently demonstrated lower risk levels, providing evidence of best practices due to the strict requirements, burden for evidence and supply chain assurance that are part of the ASC certification program. The model emphasizes key impact areas (e.g. deforestation, pollution, fishing pressure) and enables companies to pinpoint risks, prioritize mitigation measures, and guide strategic decision-making. Our risk-based approach fills a critical gap in biodiversity data and provides a scalable framework for aquaculture portfolio assessment. It supports businesses in gradually improving their biodiversity knowledge and aligning with CSRD requirements.

Further pilot studies are underway to refine the methodology, including the consideration of indicator weighting and cumulative scoring mechanisms. These advancements aim to drive meaningful improvements toward more sustainable aquaculture practices.

5.11.P-Th349 Integrated Approaches to Addressing Sustainability, Quality, and Safety in Seafood Supply Chains

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Food supply chains (FSCs) face critical challenges in quality, safety, and environmental impacts, often treated as isolated issues. This siloed approach has led to policy inconsistencies and inefficiencies. Emerging management models, such as the European Food Policy, are shifting toward integrated frameworks that link production and consumption patterns with environmental and quality outcomes, but their implementation is often constrained by data gaps and stakeholder misalignment. Additionally, fraud particularly in the seafood sector adds another layer of complexity, with an estimated 30% of seafood sold globally being misrepresented or mislabeled due to limited transparency.

This lack of reliability directly impacts decision-making in the HORECA (Hotel/Restaurant/Café) sector and retail channels, where prioritizing sustainable, non-overfished species is vital for driving a wide change. However, despite the proliferation of certifications and ecolabels aimed at guiding sustainable choices, consumer trust is undermined by ethical uncertainties and doubts about the credibility of these schemes. The incorporation of a life cycle thinking approach and the use of scientifically recognized methodologies such as Life Cycle Assessment (LCA) provide a more transparent and reliable framework, ensuring that sustainability claims are grounded in verifiable, science-based data and offering consumers greater confidence in their choices.

To address these challenges, the SMART-FOODPRINT project aims to develop a robust framework to evaluate sustainability, traceability, and safety across the seafood supply chain from sea to plate using a life cycle perspective. This research will involve stakeholders among FSC to incorporate real data from fish producers and restaurant purchasing patterns to calculate indicators and combine them into a nexus index representing the overall performance of the system in terms of environmental, social, and economic performance.

The findings will illustrate a pathway to overcome the challenges of FSCs and serve as a foundation for developing traceability and certification schemes applicable to other food systems. This integrated methodology holds the potential to advance regulatory management and foster trust in ecolabeling practices, contributing to a more sustainable and transparent food sector.

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5.11.P-Th350 Integrating Two New Biodiversity Impact Pathways Into Life Cycle Impact Assessment of Food Products

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1. Background & purpose

Biodiversity's general state is alarming, and IPBES indicates that current species extinction global rate is at least 10 to 100 times higher than the average rate over the last 10 million years. Intensive agricultural practices, on which the global food system is based, are a direct factor in biodiversity collapse. Faced with this observation, several food transition scenarios have been developed, recommending that consumers be informed on food products' impacts, both on climate and biodiversity. Yet life cycle assessment, one of the chosen methods for this goal, does not capture most of biodiversity-related aspects. Extensive research has been published to bridge this gap, but few are actively implemented. This study aims to implement two new impact assessment pathways and apply them to a sample of food datasets to assess the relevance of the associated biodiversity loss drivers for food products impact assessment.

2. Methods

Chaudhary and Brooks (2018) method is implemented to better capture the variety of land use practices' intensity levels. Hélias et al. (2023) method is implemented to quantify impacts linked to natural biotic resource depletion. Those two new impact categories are integrated into the area of protection 'Ecosystem Quality' from LC-Impact which is evaluated on ten food products from Agribalyse database. Product samples represent a variety of protein rich products & include 3 meats, 3 fishing products, 1 aquaculture product, 1 egg and 2 legumes. Results are presented per kg of protein at consumer.

3. Results

Land use and natural biotic resource depletion explain 66% to 98% of impacts for nine of the ten food products. For wild fishes, impact is highly dependent on fish stocks and techniques while for meat, the usual hierarchy between red & white meat is observed. Regarding ranking between products, Thunnus thynnus from the Mediterranean Sea has the highest impact followed by ground beef. Legumine have the lowest impact alongside Thunnus albacares. Aquaculture shrimps show similar impacts to chicken and pork. Several limitations are identified and should be addressed in future work.

4. Conclusion

This study provides an insight on the contribution of land use and biotic resource depletion to food products' impacts on 'Ecosystem Quality'. Results suggest that these pathways can hardly be neglected and argue for a better integration of these aspects.

5.11.P-Th351 Environmental Footprint Method Aligned Product Category Rules for Food Products

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The European Commission's Environmental Footprint (EF) initiative and the Finnish LCAFoodPrint project aim to enhance reliability in communicating environmental footprints. To improve the coherence and comparability between assessment of all main food product categories, the project develops Food-LCA guidance based on the EF method, yet with some exceptions. The guidance specifies the EF method further for the agricultural sector related to global warming potential, water scarcity and eutrophication.

For land use and land use change (LULUC) emissions, the emissions from mineral and organic soils were more clearly distinguished compared with the EF method due to the significant role of organic soil emissions in Finland. Furthermore, the possibilities to utilize open-access country-specific LULUC data from national GHG inventories were improved. We do not support simultaneous calculation of LULUC emissions and Carbon Opportunity Cost due to potential double counting. For animal and crop modelling, the use of country-specific methods from the national GHG inventories was prioritized, providing that the calculation method and all needed parameters are open-access.

The use of good-quality primary activity data, as well as good-quality secondary data, was seen an important for reliable and harmonized LCA. The data quality requirements of the EF method were seen strict, but highly needed. Thus, the requirements of the EF method were adapted, but not relaxed, to be applicable for the large and heterogeneous product group of the Food-LCA guidance.

The use of the Circular Footprint Formula (CFF), as presented in the current EF version, was seen complex and even hindering the aim of harmonizing LCAs. The credits for incineration and substitution of virgin materials are seen as elements of consequential modelling that are not suitable for attributional LCA

aiming to harmonized consumer communication. The credits on incineration also make the LCAs significantly more complex, and most likely will lead to double counting. Therefore, a more simplified assessment of wastes based on allocation is proposed in the guidance.

In the project, also a guidance on communication was developed.

The developed specifications of the Food-LCA guidance could be considered in the future revisions of the EF method. To allow improved comparability between larger product categories, it is seen important that the PCRs of at least similar product categories align in the future.

5.11.P-Th352 Comparing results for Life Cycle Assessment and Ecosystem Services - Exemplified by Dairy Production

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The sustainability of agriculture is becoming an increasing concern for the general public. In this context, Life Cycle Assessments (LCA) are gaining importance. At the same time, it is recognized that agricultural land provides multiple important functions and services. The relationship between LCA and Ecosystem Services is increasingly discussed in recent LCA literature. Therefore, we investigate the relationship between indicators of Life Cycle Impact Assessment (LCIA) and Ecosystem Services in the context of dairy production.

To this end, we performed an LCA for 89 dairy farms with a functional unit of 1 ha managed farmland. The indicators assessed are Global Warming Potential (GWP), Marine Eutrophication Potential (MEP), Terrestrial Acidification Potential (TAP), Fossil Resource Demand (FRD) and Land Use (LU). Simultaneously, we assessed ecosystem services provided by agricultural land managed by these farms. The ecosystem services selected for assessment include food production, drinking water potential, erosion prevention, plant biodiversity and recreation potential. Data were collected by an online survey, from the Integrated Administration and Control System (IACS), freely available GIS data, remote sensing, and literature.

Preliminary results indicate that LCIA indicators correlate strongly when assessed for the functional unit of 1 ha. Strong negative correlations are observed between drinking water potential and all LCIA indicators, as well as between TAP and FRD and plant biodiversity and recreation potential. Erosion control, on the other hand, correlates poorly with the selected indicators. Overall, these negative correlations paint a similar picture for both LCIA and ecosystem service indicators: High production intensity per ha in dairy production is associated with higher impacts and lower ecosystem services. To improve comparability, it is necessary to harmonise the system boundaries and functions of the production system under consideration. Harmonisation of these aspects between LCA and ecosystem services remains a challenge and needs further discussion.

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5.11.P-Th353 A Sustainable Transportation System Will Require Sustainable Dietary Patterns and Food Systems: Focus On Cycling Life-Cycle Impacts Including Additional Calories Intakes and Regional Diet Evolutions

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Increasing active mobility cycling, walking seems a promising lever to mitigate impacts to climate change, but switching to active modes requires to produce more food to supply additional calorie intakes (ACI). Accounting for more than 1/3 of the global land distance traveled by passengers, cycling also requires substantial extra calories, making its environmental impacts intrinsically linked to food system s sustainability. This study aims to explore (a) the life cycle impacts of different cycling practices by region and type of bike including ACIs, and (b) the share of cycling environmental impacts in prospective mobility scenarios under different archetypical regional diet evolutions.

The method is based on : (1) modeling scenarios of global future transportation demands for land passenger travel, (2) conducting the LCA of transportation modes, (3) assessing the environmental impacts of the scenarios. (1) is an extrapolation of the International Transportation Forum scenarios, where technologies by mobility mode are completed using other sources. In (2), we develop regional archetypes of conventional modes to conduct prospective LCAs using premise. We gather existing and develop new life cycle inventories of bikes (electric/mechanical (e-/m-), standard/sportive/cargo, aluminium/bamboo/carbon), model calories consumed depending on regional cycling practices

(speed/intensity) using the Metabolic Equivalent model, as well as different dietary patterns (vegetarian, keto, 6 average regional diets), to calculate the life cycle impacts of different cycling practices and transportation scenarios using IMPACT World+ (3).

Cycling environmental impacts change highly when considering ACI. The carbon footprint of cycling in Africa goes from 11 to 26 gCO₂e/km, while at the other extreme, it jumps to 95 gCO₂e/km in Europe. Under a keto diet, cycling with a m-bike emits 135 gCO₂e/km, what is like using an e-car with a green electricity. Under highly emitting diets, e-bikes perform better, but e-biking or m-biking emits similarly under a vegetarian diet (35 g). Under stable diets and food systems but high decarbonization ambitions in the transportation sector, cycling would reach by mid-century 1/6 of the contributions of the global land transportation to CC and water scarcity, and 1/3 to its land occupation and marine eutrophication, mostly due to ACIs. Becoming all vegetarians mitigates some impacts but may make the contribution to water scarcity explode.

5.11.P-Th354 Replacing Meat and Dairy with Plant-Based Options in Spain Could Reduce Environmental Human Health Damage by a Third While Promoting More Nutritionally Adequate Diets

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The shift from traditional plant-based Mediterranean diets to Western dietary patterns in Spain has exacerbated chronic diseases and increased environmental degradation. This study quantifies the human health burden linked to the environmental impacts of Spain's food demand, assessing the potential benefits of reducing meat and dairy consumption and food waste. Using the IMPACT World+ v2.0.1 Life Cycle Assessment (LCA) method, the environmental health impacts of food consumption and waste in Spain during 2022 were evaluated. Scenarios involving the reduction or replacement of meat and dairy with plant-based options and the mitigation of food waste were analyzed for environmental human health and nutritional outcomes. The ReCiPe 2016 v1.1 method was used for sensitivity analysis. Meat, fish, and dairy contributed 56% of dietary environmental human health damage, with total food demand resulting in 228,156 DALYs. Replacing 100% of meat and dairy with plant-based alternatives could reduce this burden by 32.4%, while eliminating food waste could lower it by 4.6%. Combined interventions may prevent 37% of DALYs. Nutritional analyses demonstrated that these dietary changes would result in diets more closely aligned with WHO guidelines, promoting improved adherence to recommended nutrient intakes. Transitioning towards plant-based diets and reducing food waste can significantly decrease environmental human health damages while fostering healthier, sustainable dietary practices in Spain. Policymakers should integrate these strategies into public health initiatives to mitigate climate and health crises.

5.11.P-Th355 Life Cycle Assessment of an Innovative Agrivoltaic Apple System and Comparison with Conventional Apple Production

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The cultivation of apples represents a highly relevant agricultural sub-sector in Austria. Nonetheless, a number of challenges currently exist, including rising temperatures and extreme weather events resulting from climate change, which can lead to lower yields and poor quality. Additionally, new pests and diseases have emerged due to altered production conditions alongside economic considerations, including fluctuating market prices and intensifying market competition. One potential solution to protect apples against extreme weather conditions, potentially decrease pesticide use and also to provide additional income in form of sold electricity, is the combination with electricity production by stilted PV modules: agrivoltaics. In this study, an apple agrivoltaic system in Lower Austria is assessed using the Life Cycle Assessment method to evaluate potential environmental impacts and compare them with the current status quo of conventional apple production. A cradle-to-gate Life Cycle Assessment is conducted based on primary plant data for both the innovative and the conventional apple production. As the agrivoltaic system under study is a multi-output system, it is necessary to consider how the multi-outputs should be dealt with. In order to comply with the allocation hierarchy, set out in ISO 14044 and to facilitate the communication of the results to a broad audience, inputs and outputs are divided between the two outputs apples and electricity. In the case of inputs such as the mounting structure of PV modules, the division is based on the wood pillars for hail nets in conventional production. The functional unit is set to 1 kg of apples and 1 kWh of electricity. The most important impact categories, including climate change, eutrophication, acidification and toxicity, will be assessed using ReCiPe Midpoint 2016 impact assessment method. The software openLCA v2.1.1 with Ecoinvent database 3.10 will be used. The overall aim of this

work is to evaluate the advantages of this innovative apple production system compared to a conventional production.

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5.11.P-Th356 Environmental Sustainability of Diets for Fish and Poultry Production and Links With Animal Health

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The growing global population has increased the demand for fish and poultry products, species valued for short production cycles, and high feed efficiency. The Life Cycle Assessment (LCA) methodology has been applied to quantify the environmental impact of fish and poultry production systems, identifying feed production as the primary contributor to global warming impacts. This approach supports adopting sustainable animal feeding practices to mitigate these environmental effects. This includes improving the feed efficiency of the animals and incorporating alternative and locally produced feed ingredients (e.g., insect-based products, algae, etc) into diets. Ensuring the environmental sustainability of fish and poultry production through animal diets requires meeting the animals' nutritional needs and reducing their environmental processing impact while supporting their health and welfare. This review aims to assess the environmental performance of diets in fish and poultry production based on LCA while also examining their connection to animals' health impacts. For this purpose, articles published in peer-reviewed journals focusing on LCA of diets for fish and poultry were considered. The final sample encompasses 56 case studies and 4 reviews. Different involved scenarios were identified within case studies as 103 occurrences, corresponding to 78 LCA and 25 nutritional adequacies for the animals. This work shows that LCA methodological choices varied among the case studies, namely in selecting the functional unit, system boundaries, and multifunctionality. Global warming (measured in kg CO₂ eq.) is the most frequently assessed impact assessment category. Notably, crop production (e.g. soybean) and fishery by-products were identified as major contributors to the overall environmental impact of feed production. Regarding animal health impacts, the diet formulations analysed were generally comparable to reference diets and aligned with established dietary requirements. Further investigation is needed to advance toward a holistic approach, recognizing the interconnectedness of animal and human health, while ensuring sustainability. This holistic perspective should prioritize food security, promote sustainable resource use, and support a circular economy.

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5.11.P-Th357 Framework to Conduct a Life Cycle Sustainability Assessment for Insect-Derived Products

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The increasing demand for sustainable food and feed products has driven the agri-food and livestock industries to seek innovative alternatives, with insect-derived products emerging as promising nutritional sources that support circularity. To comprehensively assess the sustainability of these products, a Life Cycle Sustainability Assessment (LCSA) framework was developed, defining methodological choices for environmental, social, and economic assessments. This latter component replaces the traditional life cycle costing analysis that focus on costs. Integrating an economic assessment, both costs and economic impacts through the supply chain are encompassed in LCSA. The Life Cycle Assessment (LCA) is grounded on the Environmental Footprint (EF) guidance, while the Social Life Cycle Assessment (S-LCA) follows UNEP/SETAC guidelines. The proposed framework promotes the harmonization of shared elements

across the four interrelated phases of the life cycle structure for the three methodologies, supporting stakeholders in the agri-food industry in informed decision-making. No specific recommendations for insect production or insect-derived products exist. Therefore, for the LCA, it was recommended to assess the sixteen impact indicators and select the more relevant ones following the EF guidelines. In the S-LCA and economic assessments, the amount of existing impact indicators combined with limitations in data availability creates challenges in assessing all of them. In this sense, a procedure for selecting impact indicators has been developed, to ensure that the perspectives of relevant stakeholders -workers, local community, society, consumers, and value chain actors- are considered. Moreover, the impact assessment methods selected are the Reference Scale approach for S-LCA and the monetary valuation for economic assessment. This framework also describes how to identify environmental, social, and economic hotspots. Future research should focus on the validation of these methodological approaches, to enhance the framework's applicability as the insect industry continues to grow.

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5.11.P-Th358 Insect Frass as a Sustainable Alternative to Conventional Fertilizers: Enhancing Soil Health and Agricultural Productivity

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The increasing need for sustainable food production has driven research into novel organic fertilizers, such as insect frass, an entomofertilizer resultant of insect farming. This study explores the use of frass from *Hermetia illucens* (black soldier fly) larvae as an alternative to conventional synthetic mineral fertilizers, NPK (nitrogen, phosphorus, potassium), associated with nutrient hotspots and eutrophication events. Two different frasses were used: 1) frass obtained from insect digestion of cereal waste and 2) olive-pomace frass, an entomofertilizer resulting from the bioconversion of olive pomace, a residue of the olive oil industry. The aim of this study was to assess the potential of both entomofertilizers to replace or complement mineral fertilizers in agriculture at a laboratory scale. In addition to the control group, which consisted of natural LUFA 2.2. soil with no added fertilizer, the treatment groups included both mineral fertilizer and each type of frass at relevant rates, and different ratios between NPK and frass application. Plant performance endpoints were assessed (germination, biomass, photosynthetic activity and productivity) in two full life cycle plant pot tests with *Brassica rapa*, following the ISO 22030 guideline. In the first cycle, all fertilization regimes were applied to the soil. For the second full life cycle plant test, the soil that remained after harvesting was used without further amendment. Soil function was assessed by measuring soil enzyme activities (urease, α -glucosidase, aryl sulfatase, dehydrogenase, and phosphatases) at the end of each cycle. Additionally, to evaluate the safety of all used fertilization regimes, ecotoxicological assays were performed using two soil model invertebrates, *Enchytraeus crypticus* and *Folsomia candida*, following the OECD-220 and OECD-232 guidelines, respectively. Results showed that all fertilization regimes increased significantly plant performance endpoints. Frass induced soil enzymatic activity, especially at the end of the second plant cycle. This reveals a long-term positive influence on soil biochemical processes and nutrient cycling. Survival and reproduction of *F. candida* were not affected, while reproduction of *E. crypticus* increased significantly with organic fertilization. This study highlights the efficacy and safety of insect frass as a promising solution for sustainable agricultural practices.

5.11.P-Th359 Life Cycle Assessment of Insect-Derived Frass: Perspectives from a Literature Review

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One conceivable approach towards sustainable development and circular business models within the agri-food industry is using insects to convert biomass into alternative protein sources. Besides protein, insect production also generates frass, composed of insect excrement, exuviae, and leftover feedstock, viewed as a valuable product that holds potential in the fertiliser sector, due to its nutrient content. Life cycle assessment (LCA) can be applied to identify the environmental benefits and drawbacks of insect-derived

products, including frass. However, this can be challenging from several perspectives (e.g. multifunctionality) and, so far, no harmonised method is available.

This study aimed to conduct a literature review using Scopus and Web of Science to assess LCA studies that include frass production and/or application. The search was performed using combinations of keywords and their abbreviations, including: life cycle assessment OR carbon footprint combined with frass OR insect AND fertiliser OR compost. A total of 17 studies were analysed, focusing on aspects such as the goal, system boundaries, and multifunctionality.

The goal of the studies spanned from assessing insect-derived products (primarily focused on protein) or comparing waste management technologies. While system boundaries exclude the use stage in most studies, some include frass application, with varying assumptions on emissions. Most studies consider that frass substitutes fertilisers based on the respective nutrient content (considering one or more nutrients), assuming equal nutrient bioavailability regardless of the fertiliser considered. However, some studies are unclear on the assumptions for fertilizer substitution. For example, the avoided fertilizer type is not identified.

In conclusion, increasing the transparency of the studies is recommended, as well as harmonising the methodological choices to guarantee study comparability. Future research should consider findings from interdisciplinary research (e.g. soil science) to improve the quality of the assumptions and overall impact assessment.

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5.11.P-Th360 Toxicokinetics of Polycyclic Aromatic Hydrocarbons (PAHs) Accumulation in *Hermetia illucens*

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Entomoculture industries widely use *Hermetia illucens* larvae due to their exceptional ability to consume a wide variety of organic matter. Also, the European Commission approved the consumption of *H. illucens* for animal feed in 2017. Therefore, the role of *H. illucens* in waste management and protein production raises concerns about introducing contaminants from waste sources into the food supply chain. The presence of polycyclic aromatic hydrocarbons (PAHs), known as oncogenic substances, in animal feed remains unregulated despite their restriction to human consumption. Considering the need to understand how and up to what extent PAHs accumulate in edible insects, this study aims to fill this gap in scientific knowledge. For that, a two-phase bioaccumulation assay with toxicokinetic modelling was conducted using four different PAHs (benzo(a)pyrene, Benz (a)anthracene, benzo(b)fluoranthene, and chrysene) given to insects in the substrate (Gainesville (GV) diet, consisting of 50% wheat bran, 30% alfalfa, and 20% corn meal). The experiment was divided into two phases: 5 days of exposure to the contaminated substrate in the concentration of 12.5 µg kg⁻¹ for each PAH, according to the EU limitations for undesirable substances in food - 74PAH 50 µg kg⁻¹. The subsequent phase was transferring the organisms into the non-contaminated substrate for 5 days. During the experiment, five replicates of organisms were sampled each day, and the concentration of PAHs in insect bodies was measured. The Akaike Information criteria (AIC and AICc) identified the one-compartment first-order model with an inert fraction (FI model) as the optimal model for fitting our bioaccumulation data. The modelling identified the constant uptake rate, constant elimination rate, half-life of the contaminant, and the inert fraction. Accumulation occurred in chrysene, benz(a)anthracene, and benzo(b)fluoranthene, but the only PAH that we were able to apply toxicokinetic modelling was benz(a)anthracene. However, the concentration of PAHs in insects was higher than the EU limitation on the last day of exposure to the contaminated substrate. On the other hand, the half-life of the contaminant was less than one day, expressing the ability of organisms to eliminate the contaminant. These findings underscore the importance of a depuration phase in entomoculture industries, particularly when they utilize agricultural, agro-industrial, and other organic waste streams for insect production.

5.11.P-Th361 Crickets and Metals: Uptake and Elimination of Four Metals in the House Cricket *Acheta domesticus*

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Among the four legal insect species for human consumption in the EU, the house cricket *Acheta domesticus* is the second most studied, primarily under ecological perspectives rather than food safety. *A. domesticus*, like other insects, can be reared on various unused organic matter, promoting a circular, sustainable economic model. Regardless, ensuring food safety is critical, and there is still a wide knowledge gap in the transfer and accumulation of contaminants from substrates to *A. domesticus*. With that in mind, this study aimed to evaluate the uptake and elimination of four metals considered of concern: arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) by *A. domesticus* nymphs, using a toxicokinetic approach.

The study included a ten-day uptake phase, where 10-day old nymphs were fed metal-spiked chicken feed, followed by a ten-day elimination phase with clean feed. Metals were introduced as HgCl₂, CdCl₂, NaAsO₂, and Pb(NO₃)₂ at regulatory benchmark concentrations for feed (0.1 mg/kg Hg, 2 mg/kg Cd and As, 10 mg/kg Pb, dry weight). Control groups were fed with clean food only in both phases of the test. Conditions included a 16h/8h light/dark cycle at 28±1°C. Samples were collected every other day, lyophilized, and analyzed for metal content by inductively coupled plasma mass spectrometry.

Our results demonstrated that the accumulation of metals in *A. domesticus* is dependent on the specific metal. Importantly, none of the four tested metals exceeded the regulatory threshold limits for feed in the EU, with Cd having the highest bioaccumulation factor (BAF = 0.864) and Hg the lowest (BAF = 0.003). Furthermore, organisms eliminated most accumulated metals within the first twenty-four hours after exchanging contaminated for clean food, underscoring the safety of using these organisms as food and feed sources. Results indicate that *A. domesticus* accumulates metals as expected, however, to a much lesser extent than other previously tested insect species (e.g., *Tenebrio molitor* and *Hermetia illucens*). This study supports *Acheta domesticus* as a sustainable bio converter, specifically for biomass containing trace metals. Notwithstanding, it is crucial to continue expanding the scope of studies with these organisms since, as far as we know, bioaccumulation and toxicokinetic studies with other contaminants are either limited or nonexistent.

5.11.P-Th362 Understanding Contaminant Safety Across Edible Insect Species: A Step Toward Sustainable Food Systems

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Edible insects are a promising solution to meet global protein demands sustainably. However, concerns about contaminants like metals, PAHs, and mycotoxins in insect biomass raise critical questions for food safety. Ensuring the safety of insect-based foods is essential to gaining consumer trust and shaping clear regulatory frameworks. This study investigated how contaminants behave across *Tenebrio molitor* (mealworms), *Hermetia illucens* (black soldier fly), and *Acheta domesticus* (house crickets). Insects were reared on substrates with controlled contaminant levels (aligned with EU maximums for feed), assessing bioaccumulation and its implications for human and animal consumption. Two-phase bioaccumulation assays and toxicokinetic modelling were performed to estimate uptake and elimination rates, bioaccumulation factors, and contaminant residence times. This approach provided valuable insights into how contaminants are retained and eliminated in insect tissues, addressing a significant knowledge gap. Results showed species-specific differences in metal accumulation (As, Cd, Pb, Hg). Crickets exhibited the lowest accumulation, Black soldier flies the highest, and mealworms the intermediate. PAHs accumulated less than metals, but notable trends appeared in mealworms and black soldier flies.

Elimination rates were rapid for most contaminants, with near-complete elimination within one day. A critical exception was cadmium in black soldier flies, which showed prolonged retention, raising safety concerns and attention to a need for a prolonged elimination period. Exposure to the maximum allowed feed contaminant levels revealed that, in some cases, insects require short depuration periods to ensure their safe use as feed. PAHs, which are not currently regulated in feed, showed potential for accumulation, emphasizing the need for further research given their importance in food safety legislation. This work highlights the need for species-specific safety assessments and tailored farming practices to minimize risks. Within this work, we provide data for developing robust safety guidelines by addressing these challenges. Our findings contribute to integrating edible insects into sustainable food systems while ensuring their safety and nutritional value for consumers worldwide.

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5.11.P-Th363 Chemicals of Concern in Food Packaging: A Matter of Sustainability

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In a globalized system, food is increasingly processed and transported over long distances. This is enabled by highly functional food packaging. However, food packaging is also a driver of long-term damage to the environment and human health. From the sourcing of raw materials over production to end-of-life, food packaging has multiple impacts on the use of resources, environmental pollution, and exposure to chemicals. Therefore, decision makers in the food sector need transparent and science-based information to choose safe and sustainable food packaging for each type of food.

The UP Scorecard was developed for this purpose and is built on a scoring system, including the six impact areas climate change, water use, plastic pollution, sustainable sourcing, recoverability, and chemicals of concern. While other packaging assessment tools are available, they often focus on a single impact area, such as climate change, but usually omit the chemical safety of a material.

To assess chemicals of concern within the UP Scorecard, we applied a hazard scoring system to over 14,000 known food contact chemicals (FCCs), based on the methodology described in the PlastChem report (DOI: 10.5281/zenodo.10701706). We focused on the human health hazards only, as there is direct oral human exposure from the many chemicals that migrate from packaging into foodstuffs. In total, we found 4680 FCCs with hazard scores indicating different levels of concern for human health. 419, 121, and 406 out of these FCCs have the highest hazard score for carcinogenicity, mutagenicity and reproductive toxicity, respectively, whereas 45 FCCs are confirmed endocrine disrupting chemicals, and 1330 FCCs have specific organ toxicity.

To facilitate access to this information for decision-makers, such as procurement professionals, we grouped the chemicals of concern based on their structural properties but also integrated filtering options regarding the use and presence of FCCs in food contact materials. This will help to identify, screen and avoid the most hazardous chemicals for each food packaging material and support development of safe and sustainable food packaging solutions.

5.11.P-Th364 Exposure to Micro- and Nanoplastics: Is Food Packaging a Source?

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Micro- and nanoplastics (MNP) have been detected in a variety of foods and beverages, and concerns for MNP impacts on human health exist. Environmental contamination has been identified as one exposure source of MNPs to foodstuffs. However, the role of plastic food packaging or other plastic food contact articles (FCA) has received less attention despite plastics being ubiquitously used in the food system.

Therefore, we systematically mapped the scientific evidence of MNPs present in foodstuffs and originating from FCAs during normal and intended use. We searched several public databases for relevant studies, and, from the eligible ones, we extracted a wide range of data pertaining to experimental design, FCA type, MNPs properties, and the food type or food simulant. In addition, we critically appraised the included studies regarding general data quality, material identification methods, polymer type reporting, and study design, using a newly developed method. The extracted data can be accessed through a publicly available dashboard

We identified 104 studies matching our eligibility criteria. From these sources, we extracted data and generated 601 database entries. The most studied FCAs are bottles (31% of all entries), containers (19%), tea bags (12%), cups (10%) and bags (10%). A total of 212, 121, and 257 entries refer to the analysis of MNPs in liquid and solid foodstuffs as well as food simulants, respectively. While the included data suggest widespread contamination of MNPs in food over 90% of entries reported an MNP presence most studies are of low quality. Using our critical appraisal, we found only seven studies to be highly reliable. These studies demonstrate that under intended or foreseeable conditions of use, plastic FCAs can release MNPs into foodstuffs. Importantly, our reliability assessment also emphasizes that a harmonized approach

to testing and reporting MNP migration from FCAs into food is needed to improve data quality and reporting, and to ultimately introduce mitigation measures for reducing human exposure to MNPs.

Given the current knowledge gaps, further research to characterize material- and use-dependent MNP migration. Furthermore, we propose the inclusion of MNP migration testing in regulatory frameworks for FCAs as one measure to reduce MNP exposure via food ingestion. This is especially relevant since foods are increasingly (ultra-)processed, and due to processing, the foods contact with plastics is also increasing.

5.11.P-Th365 Circularity in the Food System: Exploring the Sustainability of Alternative Uses of Whey Side Streams

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Roughly one third of food produced is wasted, either at the production, processing, or consumer level. One important subset of food waste is protein-rich side streams from industrial food processing, such as whey from cheese production. Whey contains proteins (e.g., beta-lactoglobulin) which have unique properties that are currently explored for valorization into advanced materials such as bioplastics and CO₂ capture material. The aim of this study is to assess the potential benefits and environmental impact of diverting whey from traditional uses (i.e., as animal feed) to select emerging alternative uses currently at or near the lab-scale level, namely in bioplastics for food packaging, CO₂ capture material, and wastewater treatment material. We conduct a first-order assessment assuming all whey in Switzerland (1.3E+6 tons wet weight) is diverted to one of each of these three alternative uses, estimate the total mass of whey protein-based materials that could be produced based on literature-derived conversion factors, and assess the possible uses of these materials. The potential amount of bioplastic film derived from whey would be 7 times the total Swiss demand for food film packaging, the potential mass of CO₂ capture material could capture 21% of annual Swiss CO₂ emissions, and the potential amount of wastewater treatment membrane could absorb roughly 7500 times the amount of ibuprofen present in Swiss wastewater (note this assumes extrapolating lab-scale efficiencies of the materials to real-world operation). We select the CO₂ capture material for additional investigation in a refined Swiss whey usage scenario, due to the importance of advancing carbon-capture technology in the near future. We construct two alternative whey usage scenarios: 1.) The Swiss whey currently utilized for low-grade animal feed (5.87E+5 tons) is diverted to produce CO₂ capture material, with this low-grade feed whey being replaced by a plant-based feed, and 2.) All Swiss whey used for animal feed (low- and high-grade, 9.84E+5 tons) is diverted for use in CO₂ capture material, while a nutritionally-equivalent mass of plant-based protein replaces the animal meat for human consumption that resulted from the whey feed. Ecoinvent will be used to assess environmental effects resulting from the baseline and alternative whey usage scenarios. This study provides a preliminary framework for informing large-scale decision making around the more circular use of food waste side streams.

5.11.P-Th366 Upcycling Agricultural and Industrial Side-Flows for Food Development: A Risk Assessment Approach

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Food waste and losses along the supply chain represent critical challenges to sustainability on global and national scales. Up to one-third of all food produced globally is wasted, leading to reduced producer profitability, diminished food security, and significant environmental degradation. In Sweden, primary production and food industries face substantial losses of raw materials, as identified by the Swedish Board of Agriculture. Approximately half of slaughterhouse by-products and a third of carrots intended for human consumption are diverted to alternative uses such as biogas production or animal feed, rather than direct food applications. Tackling these losses aligns with the goals of the Swedish Food Strategy, which prioritizes resource efficiency, profitability, self-sufficiency, and environmental sustainability. Utilizing by-products for novel food development, however, is hindered by concerns regarding potential contamination. Environmental contaminants such as dioxins, furans, PCBs, PAHs, PFAS, plasticizers, pesticides, pharmaceuticals, and trace elements can enter the food chain posing risks to human health and complicate regulatory compliance, emphasizing the need for robust chemical analysis and risk assessment. This study explores upcycling opportunities for agricultural and industrial side-streams in Sweden, focusing on both plant-based and animal by-products. Through literature reviews and stakeholder workshops, we identified promising candidates for chemical analysis and food development, including pig and beef blood, beef kidneys, carrots, and apple press residues. Chemical analyses were conducted to assess the above-mentioned contaminants, following rigorous protocols with microwave extraction,

ICPMS, LC or GC-MS/MS, suspect and target analyses. Nutritional profiling revealed that these by-products are nutrient rich, with high protein, vitamin, and mineral content making them viable for food innovation. Our findings highlight the dual potential of these side-streams to reduce food waste and develop sustainable, high-value food products. The study underscores the importance of integrating risk assessment into food innovation to ensure safety, enhance market acceptance, and support circular economy objectives, while also contributing to addressing critical sustainability issues by converting underutilized resources into nutritious and safe food alternatives, fostering resilience within food systems while mitigating environmental impacts.

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5.11.P-Th367 RNAi: A Targeted Pesticide Technology for a More Sustainable Agricultural System *Laurent Mezin, GreenLight Biosciences, United States*

The global use of pesticides in agriculture and other use sites is not sustainable. Conventional pesticides affect not only the target pests but also non-target organisms, potentially decreasing biodiversity on- and off-field. The pesticides and their residues can have a detrimental effect on human health and the environment, sometimes for years after application. New technologies are needed to ensure a safer and more sustainable global food system. RNA interference (RNAi) is one such technology. This technology uses double stranded RNA as an active substance (AS) to interfere with the production of a protein in the target pest and can be designed to be very specific to a single target pest, with little to no effect on humans or non-target organisms. New pesticide AS can be rapidly designed using bioinformatics to ensure the mode of action is specific to the target pest and to avoid any likelihood of toxicity to humans or any non-target organisms. These AS are effective at low application rates, minimizing the quantity of chemicals (including other formulants) applied to a field and reducing the use of old chemistries. They are also compatible with current agricultural practices. Multiple studies have shown they have no toxicity in humans, low to no toxicity to non-target organisms, and preserve biodiversity even on the treated field. The AS degrades rapidly in the environment to naturally ubiquitous nucleotides. New AS that use RNAi are registered or pending registration in the US, Europe and other countries. From the inputs into the manufacturing process to the low application rates in the field, RNAi pesticides can reduce greenhouse gas emissions substantially, compared to conventional petrochemical-derived pesticides. RNAi pesticides have the potential to increase worker safety, support biodiversity, replace older chemistries, and reduce greenhouse gas emissions related to agriculture without placing any additional burden on the grower or farmer. Thus RNAi technology as a crop protection tool can support sustainable agriculture.

Track 6. Environmental Policy, Risk Management, and Science Communication

6.01.P Exploring Earth System Boundaries and Staying within Those Boundaries for Chemical Pollutants

6.01.P-Mo449 Moving Forward with Safe and Just Earth System Boundaries for Chemical Pollutants

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The planetary boundaries concept, first introduced by Rockström et al. in 2009, has effectively communicated the dire impacts of society's actions on the biophysical systems of the Earth. The key point of the boundaries concept is that these exceedances are pushing the Earth's biophysical systems outside of safe Holocene-like conditions upon which human society is predicated. In 2009, evidence showed an exceedance of 3 of 9 boundaries; the most recent assessment indicates that several more boundaries are exceeded. The need for a boundary for chemical pollution, or more recently known as novel entities, has been recognized but has remained challenging to quantify because of the complexity of the domain the number of chemicals and the number of impacts, among other challenges.

In 2021, Rockström, Gupta et al. extended the planetary boundaries concept to define safe Earth system boundaries according to the safe operating space for the Earth's biophysical conditions. To this they added just Earth system boundaries that explicitly address the inequitable impacts experienced by populations, notably humanity, today and for future generations. These boundaries consider global and regional

impacts to acknowledge the wide geographic variability in the severity of effects. Thus, a global boundary can be (and is) exceeded as a result of overshooting multiple regional boundaries. Importantly, this effort, under the Earth Commission 2.0, strives to build transformative pathways that will move society from current, unsustainable trajectory to new, more safe and just conditions.

Applying the Earth system boundary concept to chemical pollution continues to be a challenge. Here we present our approach for exploring safe and just Earth system boundaries for chemical pollution and charting alternative sustainable futures through transformative pathways. The approach includes three pillars. First, data mining will be conducted to scan the horizon for existing and emerging issues to avoid looking under the lamppost and to identify future trends of chemical pollution. Second, we will investigate data-rich case studies to probe exceedances of safe and just boundaries. Finally, we will develop future transformative pathway scenarios, taking into account social, economic, technological and political dynamics and interactions, that could be implemented to move society towards a sustainable future.

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6.01.P-Mo450 Outside the Safe Operating Space of a New Planetary Boundary for PFAS: An Update

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It was recently proposed that, as with planetary boundaries, safe Earth system boundaries (ESBs) should define limits for maintaining a stable and resilient Earth system, whereas just ESBs should define limits to minimize harm to humans, including harm to human health and meeting minimum access to needs for today's and future generations. In a previous paper, we hypothesized that environmental contamination by per- and polyfluoroalkyl substances (PFAS) defines a separate planetary boundary and that this boundary has been exceeded. With the separation of planetary boundaries into just and safe ESBs, we would now conclude that a just ESB for PFAS has been exceeded. In this presentation, we will revisit the previous conclusions of our paper in light of new information on safe exposure limits for PFAS. We will also consider if this methodology can be applied to other contaminants. Recently, the United States Environmental Protection Agency (US EPA) set the Maximum Contaminant Level Goal (MCLG) for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) to zero due to the evidence that these substances cause cancer. The finding that these two PFAS have no safe level strengthens our previous conclusion that a just ESB for PFAS has been exceeded. We argue that the combination of high persistence and a no threshold toxicity has led to a number of substances already breaching a just ESB in addition to PFAS. For example, there are several substances which have an MCLG set to zero by the US EPA and are known to be globally spread. In addition to PFOS and PFOA, those substances are: chlordane, 2,3,7,8-tetrachlorodibenzo-p-dioxin, heptachlor, heptachlor epoxide, hexachlorobenzene, polychlorinated biphenyls (individual PCBs and Aroclor mixtures) and toxaphene. These substances are generally captured under chemical legislation such as REACH or the Stockholm Convention. However, substances with high persistence and high long-range transport potential, but relatively low toxicity and/or low bioaccumulation potential have historically been considered of relatively low concern and are not captured by chemical regulations. For example, novel entities which are globally spread and have a poor reversibility (both due to high persistence) such as plastics and trifluoroacetic acid (TFA) have also been suggested as threats to ESBs. We conclude that a more precautionary regulatory approach based on high persistence alone is warranted to prevent future problems.

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6.01.P-Mo451 The Global Threat from the Irreversible Accumulation of Trifluoroacetic Acid (TFA)

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Trifluoroacetic acid (TFA) belongs to the subclass of per- and polyfluoroalkyl substances (PFAS) known as ultra-short chain perfluoroalkyl acids (PFAAs). Due to its extreme persistence and high mobility, TFA is widely detected in various environmental matrices, from precipitation, surface, ground and drinking water to plants and plant-based foods and beverages, soils and human blood serum. Currently, TFA concentrations are often much higher than those of other PFAS. TFA's omnipresence in the environment is due to various uses and emissions pathways from its use in chemical production to being a terminal

degradation product of chemicals containing the C≡CF₃ moiety and lack of a natural elimination process. Although TFA concentrations will inevitably increase in the future, what remains less clear is the planetary thresholds for which this accumulation may cause irreversible, ecological harm. Eco(toxicity) data is scarce and most no risk conclusions were based on aquatic studies that are nearly two decades old, without accounting for high environmental increases in TFA concentrations, unusual environmental behaviour and new scientific evidence. In this work, we analysed the scientific literature on TFA to assess if there is evidence of its exceedance of the planetary boundary thresholds as defined with three conditions set by Persson et al. Our analysis of peer-reviewed monitoring literature revealed over an order of magnitude increase in TFA concentrations across various environmental compartments post-2010, with studies expanding from a focus on atmospheric media to include new exposure media such as plant-based beverages, crops, dust, human serum, locusts, tree species, and drinking water. This growing presence of TFA raises concerns about potential disruptive effects on human health and Earth system processes that remain poorly understood, highlighting the need to address these gaps to assess whether TFA represents a planetary boundary threat. To investigate this, we examined both known environmental and health thresholds from regulatory sources and the potential effects reported in the literature that fall outside established toxicity assessment frameworks. The case of TFA being a planetary boundary threat will be presented, based on TFA's 1) increasing planetary exposure, which is 2) irreversible and accumulating due to emissions from many sources and could cause 3) long-lasting disruptive effects on human health and vital earth system processes.

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6.01.P-Mo452 A Multidisciplinary Perspective on the Role of Plastic Pollution in the Triple Planetary Crisis

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The ubiquitous presence of plastics and associated chemicals threatens ecosystems and human health. However, in many cases plastics are considered a waste problem whereas other impacts are poorly understood. To quantify our state of knowledge on the links of plastics to the triple planetary crisis of environmental pollution, climate change and biodiversity loss, we conducted a literature analysis to discuss the negative impacts of plastics and associated chemicals from a multidisciplinary perspective. We analyzed that in addition to being part of the pollution crisis, plastics impact climate change and accelerate biodiversity loss, hence aggravating the triple planetary crisis. The literature survey revealed knowledge gaps regarding the life cycle, release, fate, exposure, hazard and governance of plastics and associated chemicals, as well as links to climate change and biodiversity loss. Core pollution-related knowledge gaps include exposure to chemicals from plastics in-use, uptake from the air and fate in specific compartments, such as the cryosphere. When assessing the impacts on climate change, major impact pathways include life cycle-related emissions of greenhouse gases, alterations of the global carbon cycle and changes of the albedo. Regarding biodiversity loss, the impacts of plastics and associated chemicals on toxicity are comprehensively studied on low levels of biological organization, but rarely at ecosystem level. Further impact pathways include, amongst others, rafting of invasive species and pathogens as well as altered habitats. We also explore links to political and legal sciences, which need to be strengthened. Our major conclusions include: (i) The contributions of plastic pollution to the triple planetary crisis need to be better understood to deduce the right strategies / measures for reduction. (ii) In-use plastics may be the most relevant human exposure source to plastics and associated chemicals. (iii) Regulation, in particular of plastic additives as well as in-use plastics, is largely missing. (iv) Our analysis revealed knowledge gaps that only comprehensive multidisciplinary efforts can address. (v) Effective plastics governance should be aligned with climate and biodiversity goals. We urge bridging disciplinary expertise from natural and social sciences to achieve effective plastic governance and risk management of plastics and associated chemicals that protect the Earth, its ecosystems and human health.

6.01.P-Mo453 Combining Methods to Characterize the Planetary Boundary for Chemical Pollution

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Charting transitional pathways to move society towards sustainable and just practices is challenging especially for chemical pollution: the diversity of chemicals is vast, as is the variability of exposure to and

effects of unintended ambient mixtures and of ecosystems. In this presentation, we describe a vision on combining science-based methods to characterize the planetary boundary for chemical pollution, some very recent. The combined methods recognize that chemical pollution causes diverse effects via the transgression of local and regional boundaries, over longer terms. The challenges that this poses are addressed by a systematic approach that allows to derive conclusions on safe and just approaches in chemical management. In this approach, we consider the issue of chemical pollution vis a vis impacts on biodiversity, together representing two of the three planetary crises of this time.

We propose the following building blocks to be combined. First, we propose to employ scenario approaches. Via Shared Socio-Economic Pathway, as used in many planetary assessment contexts, the scenarios deliver chemical emission outlooks. Second, via multi-media fate modeling, the scenario-specific emissions are translated into regional steady-state concentrations. Third, via species sensitivity distribution and mixture modeling, the latter are translated into mixture toxic pressure levels. Fourth, via ecological modeling and supported by validation studies that link toxic pressure to biodiversity impacts, those are translated into damage levels, expressed in terms of biodiversity impacts. As additional step, fifth, novel methods (especially pairwise learning methodologies) for bridging data gaps are employed, given that for example hazard data lack for many chemicals*species pairs. Recently, such methods have shown that hazard data can be obtained for a very large number of chemicals.

Eventually, the stepwise methodology can characterize the distance to target of specific chemicals, chemical groups, economic activities and alternative socio-economic scenarios to the impact boundaries of chemical pollution at various spatial scales. It would also allow inverse modeling to identify which human activities and associated emissions contribute most to moving to (or beyond) the boundaries, even if the latter remain uncertain. Netto, the stepwise approach aims to provide insights to navigate a safe and just corridor for chemical pollution.

6.01.P-Mo454 Where is the Greta Thunberg for Chemicals? A Discussion on Effective Advocacy and the Importance of Youth Perspectives in Earth System Boundaries

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Climate change, biodiversity loss, and chemical pollution threaten us all; dealing with these issues should be a goal for everyone. But, as the triple planetary crisis deepens, it is the youth of today, and the generations to come, that have the most to lose. Exposure to hazardous chemicals has been linked to an unprecedented rise in cancer rates among young people, alarming decreases in fertility, and an array of endocrine disorders. At the same time, hazardous chemicals are poisoning the supplies of water and food on which future generations will have to rely.

While youth have taken up the causes of climate change and biodiversity loss, and through advocacy succeeded in generating movement on these issues, there has not been comparable activism on the issue of safer chemical management. We contend that this lack of activism stems from an absence of decision-making bodies and political fora in which youth can participate, and, more importantly, the lack of a clear vision for a future where chemicals are used and managed responsibly.

Only recently has chemical pollution begun to receive political attention on a level similar to that of climate change or biodiversity loss. As a result, work is still ongoing to promote the International Panel on Chemical Pollution, and to define the planetary boundary for novel entities. Neither effort will be easy chemical pollution is inherently complex given the wide variety of chemicals in use but both should seek to engage youth to identify what generational fairness and equity in chemicals management will look like. Young activists should be provided with dedicated advocacy platforms, like the UN Youth Climate Summit, to allow for dialogue with researchers and policymakers.

The participation of youth in policymaking will help to address the other major barrier to youth participation: the lack of a clear vision for responsible chemical management. For all its flaws, the 2 degree goal for climate change recommended by the IPCC has become a rallying point for activists, providing a clear and actionable goal for them to advocate. Establishing safe and just Earth system boundaries, even if more challenging and subjective for chemicals, should be urgently prioritized. Activist groups, and youth groups in particular, have proven to be capable of driving the discussion and securing commitments on the issues of climate change and biodiversity loss. It is time to empower them to do the same for chemical pollution.

6.02 Tackling the Triple Planetary Crisis: Implementing Lessons Learned from the Past 30 Years of Research and Regulations to Solve Present Challenges

6.02.T-01 Effective Risk Management of Chemicals in a Changing Environment

Elin Leander, Marlene Ågerstrand and Christina Ruden, Stockholm University, Sweden

The triple planetary crises of climate change, biodiversity loss, and pollution are closely linked, but the

impact of pollution from waste and chemicals on biodiversity is less studied than the effects of climate change. Climate change will further stress Baltic Sea marine life, already exposed to hazardous chemicals, due to rising temperatures, lower oxygen levels, and increased toxins from cyanobacteria. With the environment already under pressure and chemical production rising, urgent action is needed to prevent further harm to both human health and the ecosystem.

Indication that regulatory action can be effective in reducing the risk is seen in the case of regulation on pesticides and substances subject to REACH authorization, which has effectively decreased the volume of the most harmful substances put on the market. However, both regulators and researchers underline the importance of improving the EU chemicals legislation. There is a need for more knowledge on what is an effective measure or not, to better assess whether the objectives of EUs chemical policy, to protect human health and the environment, are being met.

In our project we are investigating the effectiveness of chemical management measures with the purpose of understanding if and how current management measures can be improved. For this, a series of case studies is performed evaluating management measures using data on the production and import of chemical products, time-series of biomonitoring and effect monitoring. This is mapped against the performed management measures, in order to find correlations between an intervention and an effect. The starting point is regulatory measures, such as REACH restrictions and CLP classifications and international agreements tackling pollution.

This research contributes to expanding the knowledge base on environmental management and the role of effective risk reduction of chemicals for healthy ecosystems in the Baltic Sea. This is policy-relevant knowledge that can contribute to using societal resources more effectively for protecting human health and the environment. It is important for future risk management of chemicals to be more effective in reducing the risk of contaminants ending up in the Baltic Sea and threaten the already stressed coastal ecosystems which are of great importance for climate mitigation.

6.02.T-02 Lessons Learned for Greening the Pharmacy: A Lock-in Analysis Towards Solving the Triple Planetary Crisis

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Despite efforts towards sound chemicals management, widespread use of hazardous chemicals persists globally. Blumenthal et al. (2022) proposed that the concept of lock-in allows analysis of reasons for and ways to overcome barriers to the continued use of hazardous chemicals. Here, lock-in refers to complex interactions between economic, social, technological, and political factors that reinforce the status-quo. Our study aimed to extend the understanding of lock-in by conducting a case study on the pharmaceutical industry. While the use of drugs has led to major health improvements, their widespread production and consumption also has led them to become globally pervasive in environmental media, leading to harmful impacts to non-target organisms.

Creative solutions are needed to allow for the beneficial uses of pharmaceuticals while minimizing negative impacts to the environment this is highlighted by the recent concept of greening the pharmacy by Orive et al. (2022). While some technical solutions are seemingly promising, it is unclear if the industry is undergoing systematic change towards safer alternatives. For example, approvals of fluorine-containing drugs are increasing despite concerns over PFAS persistence and potential harmful breakdown products like TFA. Thus, our study aims to understand the barriers preventing large-scale transformation of the pharmaceutical industry towards safer chemistries.

The hypothesis is that a combination of economic, social, political, and technological factors creates a "lock-in" that keeps things as they are. This system of drug design prevents the industry from making large-scale changes toward environmentally friendly practices, meaning current technical solutions will be less effective unless lock-ins are addressed.

We identified two major lock-ins. Lock-in 1 shows how misaligned environmental safety testing timelines hinder the development of safer future drugs. Lock-in 2 highlights how the industry's profitability-driven model favors new drug development over revising existing ones, creating a barrier to improving drugs already on the market.

The lesson learned is that addressing both lock-ins is essential for any technical solutions to greening the pharmacy to be truly effective. In response to this, we propose a strategic approach to identifying leverage points within complex systems which can be used as a tool for meaningful policy interventions both within the pharmaceutical industry and beyond.

6.02.T-03 A Decision Support Tool for Choosing Pesticides with Lower Risks to Aquatic Environments

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Pesticide contamination from agricultural runoff is a major contributor to poor water quality in waterways discharging to the Great Barrier Reef (GBR) lagoon in Queensland, Australia. Water samples frequently contain pesticide at concentrations that exceed the Australian and New Zealand guidelines for ecosystem protection, and a recent study identified multiple waterways draining sugarcane dominated catchments as aquatic ecotoxicity hotspots. Previous efforts to reduce pesticide inputs into aquatic environments have focused on regulating problematic pesticide active ingredients (PAIs), improving farm management practices and improving application techniques. This project aimed to provide an alternative complementary approach that informed decisions about pesticide usage. The Pesticide Decision Support Tool (PDST) was co-designed with stakeholders including government, farmers, resellers, extension officers and agronomists. Initially developed for the sugarcane industry, it includes 104 PAIs registered for application on sugarcane and rotation crops in Queensland and has recently been expanded to include 34 PAIs registered for bananas. The PDST offers generic guidance on which PAIs pose lower potential aquatic risks, through a plot of environmentally relevant parameters: the measure of toxicological effect (MoE) and the measure of mobility and persistence (MoMP). The MoE indicates the potential of a PAI to exert harmful effects on aquatic organisms, based on aquatic toxicity and maximum application rate. The MoMP indicates the potential of a PAI to move from land via runoff and to persist in aquatic environments, based on environmentally relevant physicochemical properties. The PDST presents the aquatic risk as a continuum of values, allowing users to easily identify and select less environmentally harmful PAIs to control pests. Over a two-year period, the PDST was implemented into pesticide management plans, along with improvements to spraying equipment, through the Farmacist Pty Ltd led Project Bluewater. This resulted in a 29% reduction in herbicide application to 10,500 ha of sugarcane in the Mackay Whitsunday region in Queensland, and a 53% decrease in total relative risk units. This highlights the potential for voluntary farm education programs in reducing the impact of PAIs on aquatic environments.

6.02.T-04 Stakeholder Perceptions on the Socio-Technical Factors Affecting the Use of Evidence in Europe(an Chemical Assessment and Management)

Lowenna Bethany Jones¹, Kathryn Arnold² and Charlotte Burns¹, (1)University of Sheffield, United Kingdom, (2)University of York, United Kingdom

The need for a modern, science and/or evidence-based approach to chemical safety assessment is an increasingly urgent topic of conversation and research. Academic research is one of the many sources of evidence that inform chemical assessment and management, however in practice, academic toxicity studies face challenges to their use. In recent years there has been a significant increase in attention on who is undertaking regulatory assessments, and what data are considered in decision making processes, however, little attention has been given to how evidence (including academic research) is taken up and used for regulatory decision making. Our project explores the factors as perceived by stakeholders, that challenge the uptake and use of academic research in European chemical assessment and management. A survey consisting of Likert-scale, multiple choice and free text response questions was designed to collect and understand stakeholder perspectives on the uptake and use of evidence in European chemical assessment and management. 58 individuals responded to the survey including those in Academia (N=8), Consultancy (N=10), Government (N=22) and Industry (N=14). We find that there is a general lack of consensus on the current uptake and use of evidence, whilst actors across the regulatory toxicology system hold a range of views on the utility and role of academic research for regulatory decision making. We find general consensus on what factors are considered to challenge the uptake and use of academic research. These include a broad range of social factors (i.e. social, cultural, political, and economic considerations; N=9) and technical factors (i.e. technical, scientific, and regulatory considerations; N=3). These include highly ranked factors such as, regulatory needs and requirements (57%); expertise and resources (47%); goals and demands (40%); and legislative processes (40%). We find that technical factors are often interconnected or dependent on social factors, whilst some factors exhibit both social and technical dimensions. We conclude that the development of solutions demands a coordinated, systems level approach whilst offering some immediate recommendations for actors across the regulatory toxicology system to increase the uptake and use of academic research. Though the focus of this research is on European assessment and management, we consider the factors identified to be applicable to other jurisdictions and global settings.

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6.02.T-05 The Societal Risks of Risk Assessment

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Chemical risk assessment evolved from the 50s of the last century in an attempt to control the human and ecological risks associated with the widespread production and use of chemicals. But did we succeed? Are our risk assessments beneficial to society?

In this critical contribution, I will argue, based on more than 30 years of experience in the risk assessment field, that we should redefine the role of chemical risk assessment in society. Bottom line: chemical risk assessments should become simpler, more conservative and less important.

My analysis starts by looking at the past: did our chemical risk assessments provide the protection we aimed for? There are many examples and indicators showing that chemical risk assessment currently fails to sufficiently protect human health and ecosystems. I attribute this to the chemical diversification and the inherent uncertainties in chemical risk assessment which are insufficiently acknowledged in current regulations. The uncertainties are a consequence of reductionist approaches, e.g., assessing individual compounds instead of mixtures and testing a limited set of species instead of an ecosystem. The default response to these uncertainties, i.e., more science, results in complex analyses and reports which are difficult to understand and verify by key stakeholders such as policy makers and concerned citizens. The occasional erroneous assessment (e.g., of neonicotinoids), the influential role of industry and the lack of resources and expertise for concerned citizens result in a poisonous cocktail, feeding concerns and distrust.

One of my hypotheses is that we as a risk assessment community, and as a society, put too much faith in the science underlying chemical risk assessment. This can be partly explained by (technological) optimism, resulting in unrealistic expectations of the sciences studying complex systems, such as ecology and toxicology. We should acknowledge that we do not fully understand the complexity of life. This means simpler and more conservative assessments which have the advantage of being more understandable for policymakers and concerned citizens. A first step could be the introduction of a default mixture assessment factor when allowing new substances on the market.

6.02.P Tackling the Triple Planetary Crisis: Implementing Lessons Learned from the Past 30 Years of Research and Regulations to Solve Present Challenges

6.02.P-Mo455 Key Elements of a Sustainable and Protective Chemical Control Strategy

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"This study presents findings from research on the key elements of a sustainable and protective chemical control strategy. By adhering to these elements, we can build a resilient regulatory system capable of addressing current and future challenges in chemical safety. The key elements are:

1) Comprehensive and Pro-active: A sustainable approach prioritizes long-term solutions, such as the design of non-toxic substances and products from the outset and the integration of pest management strategies. Critical to this pillar is access to robust and sufficient data that enables comprehensive hazard assessments of chemical substances.

2) Innovative and Relevant: Chemical control strategies must adapt to evolving scientific knowledge and technological advancements. This includes incorporating new data on hazards and exposures, leveraging innovative testing methods, and embracing advancements in materials science. The aim is to foster a society that incentivizes the refinement, reduction, and replacement of hazardous chemicals.

3) Efficient and Effective: The strategy relies on both enabling the market entry of safer chemicals and swiftly restricting or banning hazardous substances. Streamlining the approval process for new chemicals can drive innovation towards safer alternatives. It is essential, however, to balance efficiency with maintaining transparency, objectivity, and rigour in data evaluation and risk assessment.

4) Transparent and Objective: Full transparency in data and chemical assessments should be the standard practice. The recent EU transparency rules for food law set an important precedent that can be expanded to broader chemical regulation. A systematic approach to identifying and managing conflicts of interest is vital to uphold the objectivity of the regulatory process.

5) Enforced and Fair: A robust regulatory system must include effective enforcement mechanisms to deter non-compliance. This requires a reasonable likelihood of detecting violations and implementing sufficiently dissuasive penalties.

6.02.P-Mo456 Assessing EU's Legal Frameworks: Hazard Classification and Data Generation under CLP Criteria

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In the context of the triple planetary crisis of climate change, pollution, and biodiversity loss, addressing chemical pollution remains a critical challenge for protecting ecosystems and human health. One important aspect in addressing chemical pollution is identifying and regulating hazardous chemicals. With the Chemical Strategy for Sustainability (CSS), the EU Commission described the goal of a stronger EU chemicals legislation to facilitate the transition toward a toxic-free environment. The CSS outlines two important steps towards this goal. The first is to ensure that the CLP regulation, which implements the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) in the EU, becomes the central piece for hazard classification. The second is to make the generic approach to risk management the default for managing the most harmful chemicals, i.e., chemicals that are carcinogenic, mutagenic, reprotoxic, endocrine disruptors, or persistent and bioaccumulative. Over time, the scope also aims to include chemicals that affect the immune, neurological, or respiratory systems, as well as those toxic to specific organs. To enhance the protection of human health and the environment and to effectively extend the generic approach to risk management, the goals of the CSS emphasize the need for sufficient and reliable information generated across legislation to ensure consistent hazard identification and classification. A hazard classification enables substances to be regulated systematically based on their hazards, which allows for improved regulatory efficiency, consistent hazard assessment, and the reuse of information across borders. The later also support efforts toward a harmonized global framework for chemical management. In regards to this, we conducted an analysis of the current state of hazard assessments for the most harmful endpoints identified according to the CSS to evaluate whether the existing legal frameworks in the EU adequately supports data generation for classification according to CLP criteria. This involved a combination of document analysis of the legal frameworks and case studies of classified substances, providing an in-depth examination of the data utilized for classification and assessing whether such data could have been produced in compliance with the data requirements outlined in EU legal frameworks.

6.02.P-Mo457 Assessing Risks to Biodiversity from Exposure to Chemicals: ECETOC Task Force Findings and Next Steps

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The Kunming-Montreal Global Biodiversity Framework was adopted in December 2022 and sets out an ambitious pathway to reach the global vision of a world living in harmony with nature by 2050. Three targets of this framework (7, 14, and 15) link to assessment of risks to biodiversity from chemical exposure.

There is an increased focus in the EU on potential impact on biodiversity from chemical exposure, as evidenced by several initiatives of the European Green Deal, such as the Farm to Fork Strategy, the Zero Pollution Action Plan and the Biodiversity Strategy for 2030.

Assessing impacts of chemical on biodiversity is challenging due to the multifaceted way biodiversity is described and measured and the lack of a unified approach to quantify the effects of chemical pollution on biodiversity, especially considering the impact from other pressures such as climate change and habitat destruction.

ECETOC launched a Task Force in 2023 to review the state of the science for assessing the impact of chemical exposure on biodiversity. The Task Force: reviewed relevant regulatory and policy documents relating to chemicals to identify to what extent impact on biodiversity is already considered; gained an overview of the EU research projects on biodiversity and chemicals; and investigated biodiversity definitions and metrics used in the scientific and regulatory domains.

It was identified that biodiversity is a poorly defined term and this is reflected in EU regulatory and policy documents, whilst mention of the term biodiversity has increased in these document in the past few decades. It was further highlighted that there has been a significant and increasing number of research projects relating to chemicals and biodiversity in recent years though such research has so far not

translated into actionable recommendations. In terms of biodiversity metrics and measures, these were found to vary according to ecosystem. Among the Essential Biodiversity Variables (EBV) definitions, trait diversity and ecosystem function are the most widely applied.

The key findings from the ECETOC Task Force will be presented, along with an overview of an ECETOC workshop planned for Q4 2025 which will aim to develop an approach to assessing impacts of chemicals on biodiversity across the chemical sectors based on the current state-of-the-art knowledge and tools in biodiversity research.

6.02.P-Mo458 RE-MIX: Options to Assess and REGulate Chemical MIXtures - A New PARC Cooperation Towards Regulatory Solutions

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EU regulations are still largely focused on the assessment and management of single substances. This holds true for substance-oriented legislations such as REACH chemicals or pesticides, but also media-oriented frameworks such as the water framework directive. The need to address the co-exposure of multiple chemicals to humans and the environment has been highlighted by the European Commission's Chemical Strategy for Sustainability and is one of the identified needs of the European Partnership for the Assessment of Risks from Chemicals (PARC).

Re-Mix is starting in May 2025 as a cooperation between regulatory and academic scientists in PARC. It aims at bringing together expertise from various areas (human health and the environment). The overall aim is to support regulation by providing evidence for magnitude of mixture risks in various media, agree on assessment methodologies and tools and propose feasible options to introduce requirements for mixture risk assessment and mixture risk management in different regulations.

It will build upon previous work and include a landscaping exercise. As starting points, the generic option to introduce a mixture allocation factor (MAF) in REACH as well as its applicability for other legislations will be analysed together with case-by-case mixture risk assessments are evaluated as way forward. Further, a number of concrete case studies of the partners will substantiate regulatory solutions with evidence for mixture risks in different media, and concrete comparisons of methods and options. The project will foster collaboration between regulation and academia and EU agencies via a regular Forum for Exchange for presentation of key results which is aimed at being open for participants of other PARC activities as well as EU agencies.

6.02.P-Mo459 Co-Occurrence of Chemicals in the Environment and Their Mixture Risks – (First Results From) a Literature Review

Marius Schubert, Gabriele Treu and Enken Hassold, UBA, Germany

The widespread discharge of chemicals into the environment has raised considerable concerns about their impacts on ecosystem integrity. Despite this, continuous industrial development has led to an increasing complexity of pollution released into the environment. It is evident that multiple chemicals co-occur in variable compositions across environmental compartments, sites, and time, all of which have the potential to pose combined risks. Until recent years, ecotoxicological research, monitoring and regulatory assessments have mainly focused on single substances or substance classes. Recent advancements in the fields of analytical chemistry and risk assessment have enabled investigating the risks of chemical mixtures pollution. Monitoring studies on measured concentrations of substances in the environment are increasingly focusing on multiple co-occurring substances and calculating their potential mixture risks and effects in the environment.

In this poster, we systematically gathered available literature evaluating measured environmental concentrations of multiple substances and estimating mixture risks, with the goal of examining the current state of monitoring studies dealing with unintentional environmental mixtures in European countries. We quantify the number of studies addressing different environmental compartments, substance classes, and geographic focal points to identify gaps for future research. We also delve deeper into the subject of mixture risk assessments, evaluating the applied methodologies for environmental monitoring and estimations of mixture risk, to provide an overview of the current tools for mixture risk assessment and the magnitude of the issue. As an outlook, we summarize the current state of research evaluating observed environmental damage linked to mixture pollution. This article aims to provide a comprehensive review of monitoring-based mixture pollution research as bases for revising future risk assessment practices. Fully understanding the exposures, effects, and risks of unintentional environmental mixtures is essential for developing effective regulations to improve environmental protection, especially as current European regulations fail to meet the challenges posed by mixtures.

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6.02.P-Mo460 Screening Concept for the Hazard Assessment of Green Platform Chemicals

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Biobased platform chemicals have a potential to become a green alternative to current petro-based chemicals. It can be anticipated that the volume of biobased platform chemicals on the European market will increase significantly in the near future leading to enhanced emissions and new risk situations. Therefore, a need to comprehensively assess the (eco)toxicity of these potentially green substances is identified. The Environment Agency Austria has developed a screening concept for the hazard assessment based on (partially limited) data availability and hazardous properties of such chemicals.

For categorization and prioritization, inter alia, the REACH Regulation criteria for substances of very high concern (SVHC) and the criteria for substances of concern (SOC) from ESP-Regulation were used. Also, relevant harmful environmental properties including terrestrial and sediment toxicity were considered. The data availability was examined based on information requirements according to the REACH regulation, which were supplemented with envisaged information requirements for endocrine disruption and mobility. To reflect the new toxicity (T) criterion in the CLP Regulation for persistent, bioaccumulative and toxic (PBT) as well as persistent, mobile and toxic (PMT) substances, a screening approach was developed.

The outcome of this pilot project shows that the proposed screening concept is a suitable tool not only for assessing the need for specific additional information to fill data gaps for potential green platform chemicals, but also to categorise these chemicals into four hazard categories, depicted with traffic light symbols. These indicate the individual hazard potential and thus provide a first screening approach with respect to their relative hazards. This assessment of available data requires significant expert judgement in different fields of hazard assessment.

This work wishes to provide additional support and guidance for industry, eventually motivating for and assisting in the transition to the use of more sustainable chemicals.

6.02.P-Mo461 Survey of Green Platform Chemicals

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Despite the huge progress that chemical policy has achieved in the last years to control the risk of chemicals, fossil resource use as well as pollution by chemicals are still considered as two of the main concerns in the triple planetary crisis. Thus, the chemical strategy for sustainability considers the transition of chemical industry towards safe and more sustainable chemicals as a major challenge for future chemical policy, management and industry. For this reason, the development of safe and sustainable by design (SSBD) alternatives in the current chemical management are highly searched for. The concept of green chemistry has become an important innovation in this context aiming at the transformation of the current chemical production which is fossil based and involves substances bearing high hazardous potential. In literature the term green platform chemicals have become a key concept describing the chemical bases for such an alternative approach.

While the colour green symbolizes entities which are derived from nature in our societies and therefore genuinely considered as benign for humans, examples like phyto- and mycotoxins are in strong contrast to that picture. This cliché may also apply to green platform chemicals. Therefore, this work was aiming to investigate green platform chemicals with respect to their potential hazard based on existing and publicly available data. For this purpose, literature has been searched for the substances proposed as green platform chemicals. The identified list of substances has been further screened with respect to their hazardous properties and the regulatory activities using the European chemicals agency's website as information bases. As a promising result, 63% of the 131 surveyed substances are relatively data rich substances (being registered as manufactured/imported in volumes higher than 10 tons per year in the EU) and are of comparatively low potential hazard based on this investigation. Therefore, these bio-based substances could potentially serve as substitutes for their more hazardous and less sustainable fossil equivalents. It is recommended that the assumption of these chemicals being of low hazard should be investigated and confirmed. Furthermore, these substances should be subject to an in-depth analysis e.g. Life-cycle assessment to identify them as SSBD chemicals for industry.

6.03 Scientific Input to the New Global Science-Policy Panel

6.03.T-01 Thought-Starter on Broadening the Impact of the Science-Policy Panel through a Scientific Knowledge Holder Network

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UNEA-5 agreed on developing a new global Science-Policy Panel (SPP) for the sound management of chemicals and waste and to prevent pollution. In 2022 an ad hoc open-ended working group (OWEG) process has been initiated to prepare proposals for the panel and the delegates will meet for a resumed session (OWEG 3.2) in early 2025, back to back with an intergovernmental meeting to consider the establishment of the SPP.

The broad participation of the scientific knowledge holders in the new SPP will be key to ensuring that this panel meets its objectives and adheres to its integrative character. This broad participation should go beyond the foreseen Interdisciplinary Expert Committee (IEC) as the pressing topics of chemicals, waste and pollution prevention need support from as many relevant knowledge holders as possible and across disciplines, including natural sciences, social sciences, and humanities.

The information shared in this paper, provide the base to open up the discussion for a more holistic and systematic approach for the involvement of scientific knowledge holders in the context of the panel. The informations is based on observations and experiences at OWEG 3, Geneva, Switzerland in 2024. Building on the insights a more systematic approach of including underrepresented groups of scientific experts, disciplines as well as experts that are not yet familiar with international science-policy work could be envisaged in the future.

The observations highlight that in both, the current negotiation process under the OWEG and the future SPP, open questions persist among the SPP's target group scientific knowledge holders. These include the avenues for scientists involvement, the complexity of the political process particularly for newcomers and concerns about the understanding of scientific institutions and principles, current lack of broader expertise, imbalance of regional representation among scientists involved in the SPP. The findings provide evidence that a network flanking the panel's activities could be beneficial to the objectives of the panel. It could include the expertise of scientists who do not only come from the chemicals, sustainability, and waste sectors, but also range from social sciences, humanities to economy studies. Scientist that are new to the policy process can benefit from a network that they could approach and learn from peers.

Disclaimer/Disclosure: NB: An initiative for a network has started and operates under the name SPP Science Alliance .

6.03.T-02 The role of university research in safeguarding the planet from chemical pollution

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Universities worldwide have greatly benefited societies spanning centuries, by contributing to education and generating new knowledge. Here we highlight the pivotal role independent science conducted at universities has and continues to play in environmental sciences, focussing on environmental toxicology and chemical pollution research in terms of safeguarding the planet from chemical pollution. Independent university research produces the feedstock of innovative research and knowledge which has promoted and enabled local to global policy and regulation. For example, every global convention and policy instrument aimed at environmental protection can trace its origins back to seminal research originating from independent university research, e.g. the Stockholm Convention, or the Montreal Protocol. These contributions have come through the support of dedicated students and academics to conduct curiosity- and issue-driven inquiry to discover and understand the breadth of problems our planet faces and to pursue creative solutions. Key to this inquiry has been academic freedom and independence, as well as the provision of sufficient financial resources. Society is facing a triple planetary crisis of climate change, biodiversity loss and pollution which are closely interlinked. We argue that the role universities play in

developing solutions for healthy humans in a healthy environment this regard is acknowledged, supported and, importantly, protected

6.03.T-03 Indigenous People's Earth Observations, Knowledge and Practices in Chemical Governance. Enhancing Management and Monitoring

Yolanda López-Maldonado, *Indigenous Science, Mexico*

The regulation and governance of chemicals presents a complex challenge intersecting environmental protection, public health, and sustainable development. This paper explores the multifaceted nature of chemical regulation and governance, encompassing national laws, international initiatives, and the roles of various regulatory bodies. While existing frameworks provide some oversight, there is growing recognition that effective governance of chemicals requires an holistic and inclusive approach. The challenge lies in developing comprehensive and sustainable solutions to chemical management that integrate diverse knowledge systems, particularly those of Indigenous Peoples (IP) and local communities. These groups are often the most vulnerable to chemical contamination yet possess deep understanding of local ecosystems and sustainable practices. This study examines the importance of including Indigenous knowledge systems in the regulation, monitoring, and governance of chemicals affecting environmental and human health, including for example obtaining consent from Indigenous Peoples for any activities undertaken on their lands through the application of the Free Prior and Informed Consent form. I argue that this inclusion is not only a matter of social justice but also a strategic necessity for developing effective and equitable governance frameworks. Our approach involves analyzing existing chemical regulation frameworks, identifying gaps in current governance models, and exploring case studies where IP knowledge has been successfully integrated into environmental management practices, where such knowledge has been overlooked. Indigenous Earth Observations and knowledge can enhance our understanding of the long-term effects of chemicals on ecosystems and human communities, inform more sustainable approaches to chemical use and disposal, improve early warning systems for environmental degradation, and foster more local responses to chemical-related challenges. Findings suggest that involving communities in chemical governance can lead to more comprehensive risk assessments, culturally appropriate mitigation strategies, and improved environmental monitoring systems. Significant barriers to integration remain, including institutional resistance and lack of recognition of Indigenous rights. Results can be applied to policy development, regulatory reform, and community engagement strategies in chemical governance worldwide.

Disclaimer/Disclosure: I, Yolanda López-Maldonado, an indigenous scientist of Mayan heritage, hereby declare that I have not received any external funding, grants, or financial support for my research and scientific work. All studies and findings presented are the result of independent efforts and personal resources.

6.03.T-04 Mismanaged Plastic Waste and Interlinked Endocrine-Disrupting Chemicals in Indian Cities: Need for Effective Policy Interventions

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Plastics ending up in waste streams pose a significant challenge globally, particularly in low and middle-income countries. Among the myriad of concerns associated with plastic waste, one of the less explored yet highly consequential issues is the fate of endocrine-disrupting chemicals (EDCs) released during the burning or combustion of plastics. This study aims to investigate the fate of interlinked EDCs produced during waste plastic burning/combustion activities in India and their subsequent accumulation in local bovine milk. EDCs in plastics such as additives (phthalic acid esters, PAEs), brominated flame retardants (polybrominated diphenyl ethers, PBDEs, and polychlorinated biphenyls (PCBs) are evidenced as the primary conduits from the hotspot regions. Hotspots majorly indicate specific activities such as open burning of municipal solid waste, and informal recycling of plastic waste encompassing electronic and electrical waste. Furthermore, during the COVID-19 pandemic, the surge in plastic-based personal protective equipment (PPE) and dumped e-waste in the open dumps are the possible reasons for the increase in atmospheric PAEs, tetra and penta homologs of PCBs and heavier PBDE homologs compared to the pre-pandemic period. Prolonged environmental release of the EDCs was found to bio-accumulate in locally grazing cows, leading to increased concentrations of EDCs in bovine milk after three years of pandemic compared to pre-pandemic levels. Emerging policy frameworks including the Chemical Management and Safety Rules must account for the multi-pathway exposure to EDCs, an emerging public health concern. Risk evaluations in the Indian context are required to pave the way for elaborate monitoring and reporting avenues for efficient management and protection of the marginalized communities in India, who might be disproportionately affected by EDC exposure.

6.03.T-05 Problematisation of Plastic Pollution in the United Nations Plastic Treaty Negotiations – A Critical Interrogation of Pollution as an Issue of Environment, Waste, Economy and Health

Ellen Palm¹ and **Patrica Villarrubia-Gomez²**, (1)*Roskilde University, Denmark*, (2)*SRC, Sweden*

The severity of the plastic crisis has spurred the need for global action. In the ongoing United Nations (UN) Plastic Treaty negotiations, member states are working toward a legally binding international instrument to address plastic pollution across its entire lifecycle. However, there is no consensus on how plastic pollution should be framed as a policy problem. As with all political processes, the framing of a societal or ecological issue shapes the potential policy outcomes. This paper conducts a critical social-ecological analysis of the Plastics Treaty negotiations, employing Bacchi's feminist approach of what is the problem represented to be? . Analysing the representations of plastic pollution, rather than the problem itself, offers a deeper understanding of the issue from a systems perspective, helping to contextualise systemic challenges within policy frameworks and promote a more inclusive approach. Drawing on participant observations and ethnographic analysis from the negotiation process, we identify four key problematisations of plastic pollution: environmental degradation, inadequate waste management, economic inefficiency, and negative human health impacts. However, the dominant problematisation of plastic pollution conceptualises it as an end-of-life materials problem. Failing to include other aspects of the plastics crisis in the UN Plastics Treaty could result in a narrow and more unjust implementation, potentially exacerbating the impact of plastic pollution. More broadly, this study highlights the marginalised societal impacts of plastic pollution and contributes to the growing body of research on the power relations among actors in and around the Plastics Treaty, the plastic industry, and oil-producing member states.

6.03.P Scientific Input to the New Global Science-Policy Panel

6.03.P-Mo462 A Systems Analysis of the European Green Deal: Policy Framework and Science-Policy Interface for Global Environmental Governance

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Chemical pollution, biodiversity loss, and climate change represent interconnected global environmental crises requiring coordinated policy responses. The European Green Deal (EGD) stands as one of the most comprehensive policy frameworks addressing these challenges, offering valuable insights for the new Global Science-Policy Panel on chemical management and pollution prevention.

This study addresses the challenge of translating scientific evidence into effective environmental governance by analyzing how the EGD integrates scientific inputs into policy decisions. I examine how this framework could inform the development of similar initiatives globally, particularly in the context of the new UN Science-Policy Panel.

The research employs a systems analysis approach, combining policy document analysis, stakeholder interviews, and comparative policy assessment. I evaluate the EGD's mechanisms for incorporating scientific evidence into policy-making, focusing on chemical pollution control, circular economy initiatives, and cross-border policy coordination.

Preliminary findings indicate that the EGD's success in bridging science and policy relies on three key elements: (1) structured pathways for scientific input in policy formulation, (2) adaptive management mechanisms responding to emerging scientific evidence, and (3) integrated assessment frameworks that consider multiple environmental stressors simultaneously. The analysis reveals both strengths and limitations in the EGD's approach to science-based policy-making.

This research concludes that the EGD's framework offers valuable lessons for the Global Science-Policy Panel, particularly in establishing effective science-policy interfaces. The findings provide practical insights for developing robust mechanisms to translate scientific knowledge into actionable policy measures at the global level. This work contributes to the ongoing development of the UN Science-Policy Panel by identifying successful approaches to integrating scientific evidence into environmental governance frameworks.

6.03.P-Mo463 Defining Intrinsic Chemical Properties in the context of Global Chemical Regulations

Hans Peter H. Arp, *NGI/NTNU, Norway*

In the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), only intrinsic hazards or properties are labeled. Non-intrinsic properties are out of scope. Carcinogenicity, flammability, and molecular weight are intrinsic properties. When new hazard classes like Endocrine

Disruptors or Persistence and Bioaccumulation are introduced, their intrinsic nature must be clarified, which can be confusing due to environmental influences. This poster clarifies Intrinsic Chemical Property from philosophical and science-policy perspectives for new hazard classes, which is relevant for the Global management of chemicals.

Philosophically, an intrinsic property is one an object has regardless of context. For example, a square's shape is intrinsic, while its size or weight is extrinsic. However, debates exist about whether shapes are truly intrinsic, as their manifestation can depend on space-time context.

In chemistry, intrinsic properties are independent of the chemical's amount, such as molecular mass, bonds, melting point, and boiling point. These properties are intrinsic despite being influenced by external factors like gravity and pressure. For toxic hazards, risk is an extrinsic property. According to Paracelsus, the dose makes the poison, meaning dose is an extrinsic property of the intrinsic hazard.

Environmental classes like persistence and mobility are also intrinsic. The UN-GHS states that persistence is based on intrinsic properties, though degradation depends on environmental conditions of the receiving environment. To quote the UN-GHS: [Persistence] is "primarily based on [] intrinsic properties. However, the degree of degradation depends not only on the intrinsic recalcitrance of the molecule, but also the actual conditions in the receiving environmental compartment . Mobility, the potential of a substance to move through the environment, is like persistence, based on intrinsic properties independent of amount (size, surface charges, dipolarity), but its degree depends on environmental conditions of the receiving environment (redox potential, pH, adsorptive phases, substance concentrations).

Based on these definitions of an "intrinsic chemical properties", new hazard classes like PBT/vPvB and PMT/vPvM should be added the UN-GHS, to facilitate global management.

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6.03.P-Mo464 Considerations on Chemicals Sound Management

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The sound management of chemicals and waste is essential for protecting human health and the environment. Chemicals play an important role in our everyday lives in materials, articles and products globally. However, when not managed properly they can potentially result in adverse impacts on human health, animals, plants, ecosystems and the environment. The outcome is biodiversity loss, ecosystems degradation, climate change and the emergence and spread of infectious diseases.

For proper management of environment it is necessary to adopt, implement and enforce legal frameworks and established appropriate institutional capacities to prevent or where not feasible, minimize adverse effects from chemicals and waste throughout their life cycle.

For appropriate control it is necessary that comprehensive scientific data and information on the properties of chemicals are generated, made available and accessible for the general public.

It is necessary to give priority to sustainable solutions and safer alternatives to harmful substances in products and mixtures, including in consumer products, in their research and innovation programmes. In the area of chemicals use it is extremely important to inform end-users, consumers about the properties of chemicals, their health effects towards workers involved in chemicals use and other affected persons. It is necessary to establish requirements by law for labelling of chemicals on the properties and effects to ensure effective awareness about the properties and effects, to reduce the risks.

The objective of environmentally sound management of chemicals is to strengthen existing national structures and mechanisms for coordination for sustainable management of chemicals in an integrated manner that involves all relevant stakeholders in the implementation of the chemicals and waste conventions.

The following priorities should be addressed for making progress: the need for a higher quality of life; a lifecycle approach; a non-toxic environment; a thought set of prevention; no harm to public health; a need to act; safe alternatives and legislation.

6.03.P-Mo465 Translating Lessons Learnt from Communicating Microplastic-Related Research to Chemicals: Making the Invisible Visible

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Every scientist should engage in outreach, to make scientific outcomes more relevant and meaningful, to

educate the public, to inform policy-makers and regulators, and to make the use of tax money for research transparent. However, not all scientific topics are easy to communicate to multiple audiences. An example are chemicals and related impacts, which are in many cases invisible to the human eye, complex and intimidating, making related outreach challenging. To the contrary, research and related knowledge regarding plastics have increased tremendously during the last two decades, and so has the related outreach alongside. As a result, any person will with certain likelihood have heard about the related issues and threats. Correspondingly, researchers addressing plastics do not face difficulties in raising interest or setting the scene, whereas their challenges are directed towards conveying the right aspects and dimensions of the problem to correctly inform multiple audiences and give laypersons knowledge and tools required to contribute to achievable solutions and citizen science activities like beach cleanups. We have explored multiple ways to convey plastics-related science to fellow scientists, stakeholders, including policy-makers, regulators and non-governmental organizations, and the general public. Those approaches included cover art graphics for scientific journals, interdisciplinary stakeholder workshops from which we conveyed the major outcomes in a science comic designed for primary school children, collaboration with a multidisciplinary artist, support of the design and implementation of workshops for high school students visiting the UFZ Student Lab, contribution of exhibits to the touring exhibition OceanPlasticsLab, a blog, diverse podcasts and multiple interviews for students, newspapers, radio and TV. Here we reflect on how these multiple tools might be modified to effectively convey similar knowledge on chemicals, which are, other than plastic, largely invisible yet surrounding us in a similar way. The related exposure to and hazard of chemicals are well-established, and, last but not least, chemicals and plastics are closely interconnected, which might be a useful link for communication strategies. We particularly reflect on actions directed towards the new global science-policy panel, give an overview and personal assessment in terms of the outreach potential of different means of communication in the context of chemical pollution.

6.03.P-Mo467 Evidence and Insights From Cross-disciplinarians on the New Multi-disciplinary Science Skills and Expertise Needed for the UN Science-Policy Panel on Chemicals, Waste and Pollution Prevention

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Modern science is an international and collaborative endeavour. Scientists often work in defined and focused fields of expertise that are very important in providing evidence to inform policy development. Science evidence on the management of chemicals, waste and pollution prevention (CWP) often involves a broad team of multi-disciplinarians. Critical to success is how the new UN science policy panel (SPP) on CWP is assembled following the open-ended working group (OEWG) process, and how the panel accesses multi-disciplinary expertise through cross-disciplinary members of an Interdisciplinary Expert Committee (IEC). Through engagement activities, ongoing focus groups and stakeholder interviews, we share learnings and insights in this presentation, on the breadth of skills and expertise that will be needed by the SPP CWP.

In addition to natural science disciplines of chemistry, biology, physics, earth sciences, engineering, epidemiology, classical toxicology, health sciences, ecology, social, economic and behavioural sciences, risk assessment, also citizen science and indigenous knowledge etc., a plethora of new science disciplines are emerging and developing. For example, advanced materials, artificial intelligence, new approach methods (NAMs, in vitro, organ-on-a-chip and computational methods in toxicological sciences), improved exposure assessment using probabilistic analysis, human/ecological biomonitoring, remediation (bio)technologies, systems biology, new environmental monitoring, life cycle assessment, next generation risk assessment (NGRA) and more. Some scientists are even advocating that in a Chemicals 2.0 vision (in Europe), it is time to bypass the old gold standard methods in regulatory toxicology through a paradigm shift where animal tests are not relied upon. With foresight on the UNEP New Horizons report, we expect the Foundational Document for the SPP CWP to be agreed in OEWG 3.2 in 2025, and that assembling the

new panel with the right people round the right tables at the right times will begin in earnest. We advocate developing terms of reference for panel participation that are both inclusive and rigorous in identifying and managing all potential conflicts of interest. In the public interest, all prospective expert participants in the panel must declare transparently all their financial and non-financial interests, for a dedicated committee to review and determine conflicts of interest. Good practice on this will be shared.

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6.03.P-Mo468 Bridging the Knowledge Gap on Plastic Pellet Releases: A Case Study in the Port of Antwerp, Belgium

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Plastic pollution is increasingly threatening ecosystems health. The release of plastics to the environment is linked to various consequences for the living environment and is a major concern for policy makers. The European Commission aims to reduce microplastic releases into the environment by 30% by 2030. A regulation on preventing plastic pellet releases was proposed on October 16th 2023. However, a harmonised methodology for measuring these releases is lacking: although efforts have been made to estimate pellet release, the available data is scarce and not always based on field measurements or observations by independent parties. Bridging this knowledge gap is essential to evaluate the extent of plastic pellet releases worldwide.

We present a case study in the port of Antwerp, a large polymer hub for production, handling and distribution of plastic pellets, located in the Scheldt estuary, northern Belgium. Plastic pellets are being unintentionally released into the environment, finding their way to the Scheldt river. Measures are taken to prevent pellet releases, but the problem is not yet solved.

To identify the current extent of plastic pellet release, 57 locations on the port roads were selected in collaboration with the companies and the port authority, and seasonally monitored using manual sampling with a 50 x 50 cm quadrat. From March 2022 to February 2025, each season 5 samples were taken at each point over 2 weeks. Constructive communication with the companies and the port authority was the key to the establishment of this campaign and provided the opportunity to interact with managers and employees to implement best practices towards zero pellet loss.

Plastic pellet release is ongoing in the entire port area at an average rate of 2.7 to 4.7 plastic pellets per day per m². The results show a heterogeneous spatial distribution in the port area, variable over time. A loss of 10.6 tonnes of plastic pellets was calculated in the first year, which increased to 13.9 tonnes in the second year. Preliminary results shows a decrease in pellet release.

This attempt in estimating plastic pellet release by using long-term field measurements is a first step in bridging the knowledge gap on worldwide plastic pellet releases. The easy and feasible methodology provides opportunities to scale up or standardize as insights into the extent of pellet loss are important to provide an estimate of the current and future dynamics of pellets in the harbour and estuary.

6.03.P-Mo469 Can Unintentional Emissions in China Explain the Rapid Rise of Global Atmospheric Contamination with Hexachlorobutadiene?

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Hexachlorobuta-1,3-diene (HCBd) was listed in Annex D and E of the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2015 and 2017, respectively, mandating the global reduction of both deliberate and unintentional production. Yet, air-monitoring reveals a continuous and rapid rise in HCBd concentrations from 2008 to 2021, making HCBd one of the most prevalent POPs in the global atmosphere. The observed large scale gradients in atmospheric concentration (China >> Japan > Northern hemisphere > Southern hemisphere), in combination with large emissions of unintentionally produced HCBd reported for the Chinese tri- and perchloroethylene manufacturing sector, led us to hypothesize that Chinese emissions largely explain the observed spatial and temporal atmospheric patterns. We tested this hypothesis with a three-pronged approach, that comprises (i) a compilation of measured data on HCBd in the environment, in particular the derivation of spatial and temporal trends of HCBd in the atmosphere from data generated by a Japanese monitoring program, (ii) use of the Lagrangian atmospheric particle dispersion model FLEXPART to link concentrations measured in Japan to the history of the sampled air masses, specifically with respect to their passing expected HCBd release locations in China, and (iii) use of the Eulerian global, multimedia, time-variant Nested Exposure Model (NEM) to reproduce the observed large scale atmospheric concentration gradients and to inversely constrain time-variant Chinese HCBd emissions. FLEXPART identifies Chinese regions with chlorinated solvent manufacturing as making large contributions to the HCBd measured over Japan, whereas NEM succeeds in reproducing the

spatial air concentration gradients both between Northern and Southern Japan and between Japan and the rest of the world. NEM simulations further succeed in constraining both the size of the Chinese HCBd emissions over time and the reaction rate constant of HCBd with hydroxyl radicals. Our findings underscore shortcomings in the provisions of Article 5 of the Stockholm Convention in reducing, let alone eliminating, the environmental releases of unintentionally produced POPs, which is particular concerning given that the unintentional production of HCBd during TCE and PCE manufacturing has been known for decades. We discuss the implications for global regulatory frameworks and highlight the urgent need for improved strategies to mitigate unintentional POP emissions.

6.03.P-Mo470 Matrixing of Use Categories and Analysis on Comprehensive Risk Indicator for Each Category

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The limitations of environmental risk assessment of single chemicals have been pointed out, and discussions for the assessment of multiple chemicals are becoming more active. This is due to a general recognition that a target environment is exposed to various chemicals at the same time. It is also due to the fact that many new chemicals are being developed and manufactured day by day. For exposure assessment in the environmental risk assessment, production volumes (including imports) and emission factors are key information. In order to set emission factors for target chemical substances, information on the way of use is necessary. However, for many chemicals, especially those produced in small volumes, information on the amounts and way of use is not available. The number of the chemicals without information will continue to grow as many regulated and banned chemicals are replaced by more and more substitutions. This study focused on the categorization of use and the comprehensive risk indicators for each category in order to gain an overall picture of the environmental risks posed by chemical substances. This study consists of four steps: 1) reorganization into matrix classification (product use category and functional use category) from the detailed use category (281 category) under the Chemical Substances Control Law (CSCL) of Japan, 2) categorization of chemical substances published under CSCL for the matrixed category, 3) calculation of total production and emission amount of the chemicals under each category, and 4) analysis on comprehensive risk indicators for each category. As a comprehensive risk indicator, we used the ratio of total emissions to the average hazard concentration (HC50) for each category. As a result, several matrixed categories were picked up as higher risk.

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6.03.P-Mo471 Hidden in Plain Sight - Solvents Masking Persistent Solutes

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Persistence is a one of the key drivers for restricting chemical discharge to the environment. However, examples of substances in solvent (SiS) that are treated as a single chemical have emerged where a readily degradable organic solvent masks the persistency of the solute. Common exemplar chemicals include polymers, plasticisers and resin dispersions and reaction products with dyes suggesting that this topic will resonate across many industries.

Over the last three years, Cefas have managed the phased introduction of the SiS programme under OSPAR agreements on behalf of the UK and Netherlands to address this issue in a regulatory context and contribute to the protection of the North-East Atlantic marine environment.

Cefas observed that studies on SiS frequently demonstrated higher levels of biodegradation than when the solutes were evaluated in isolation, indicating that regulatory data submitted for SiS underestimated the persistence of many chemicals. This has regulatory and environmental implications where the persistence of a chemical is masked by its constituent components, given that persistence is an important criterion for determining whether chemical discharge to the environment should be restricted. Therefore, additional measures are required for substances in solvents to unmask their potential persistency.

This issue was raised at OSPAR's Offshore Industry Committee in 2018, and measures put in place to allow additional biodegradation data for both the solute and the solvent to be gathered from chemical suppliers. Cefas has since undertaken a 3-phase implementation programme, initially targeting high organic solvent content mixtures (>50%) and culminating with substances containing 10-30% solvent in

April 2024. A total of 37 SiS were reviewed with 60% receiving a substitution warning under the UK and Netherlands regimes because of information gathered during the SiS programme. Chemicals in each phase represent approximately one third of the total observed with most suppliers electing to assume zero biodegradation rather than undertake the testing required to obtain better characterisation.

This presentation details the processes undertaken, the impact on regulated chemicals, stakeholder response and practicalities of implementation of the programme. We also highlight the difficulties in this approach and suggest areas for further research and refinement.

6.03.P-Mo472 Overview of Current Key Projects and Research Networks Linking Chemical Pollution and Biodiversity Change in Europe

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Biodiversity loss and chemical pollution are pressing interconnected environmental challenges, yet they are often studied in isolation within distinct subdisciplines. Recent advancements in methods to assess both issues highlight the need for inter- and transdisciplinary collaboration to address their complex interactions.

In December 2023, Goethe University Frankfurt hosted an international workshop, organized by NORMAN, the RobustNature network and the German Environmental Protection Agency to bridge the international NORMAN network with the European biodiversity community. Over 12 keynote speakers and 50 participants from academia and regulatory bodies, including the European Commission's Joint Research Centre, European Environment Agency, European Topic Centre Biodiversity and Ecosystems, and the International Commission for the Protection of the River Danube, informed synthesis discussions on innovative approaches to study the interlinkage between chemical pollution and biodiversity changes.

A survey was conducted among the keynote speakers to examine trends in 13 large European projects and research networks linking chemical pollution and biodiversity changes across terrestrial, marine (50% of projects), and freshwater ecosystems (75%). This selection represents a variety of projects spanning 24 84 months and consortia involving 10 to hundreds of researchers (PIs 33%, PhD 25%, and master's candidates 21%). The projects were predominantly funded by German (36%) and EU (45%) public funding, collectively representing investments exceeding 50 million. Preliminary results indicate that most projects were inter- (64%) or transdisciplinary (18%), focusing on biology, genetics, ecology, biodiversity, ecosystem health, and chemistry (55%), as well as biological and chemical monitoring (45%). Policy and management, ecotoxicology, modeling, and data science were addressed by 18 36% of projects, while other social science disciplines, geography, public outreach, and economics were less frequently included (<10%).

These initiatives integrate innovative chemical and biological approaches to generate interdisciplinary datasets for causation modeling and societal transformation. Detailed analysis of their objectives, methods, and unique contributions will provide a comprehensive overview of state-of-the-art European research networks, regulatory perspectives, and a better understanding of the interlinkage between chemicals and biodiversity in the future.

6.03.P-Mo473 Reevaluating New and Existing Challenges That Early Career Researchers Face Across Regulatory Ecotoxicology

Lowenna Bethany Jones, Harry Bond-Taylor, Susanna Molkanen, Dylan Asbury, Rana Al-jaibachi, Thomas Malpas, Niamh O'Connor, Hana B Mayall and Grzegorz Sylwester Sowa, University of Sheffield,

A SETAC Pellston Workshop® on Improving the Usability of Ecotoxicology in Regulatory Decision-Making was held in August 2015. One outcome of the workshop was a publication, which highlighted the challenges ecotoxicologists of the future face in applying academic research to regulatory decision making. The authors propose that training students on issues of relevance and reliability will lead to improvements in the applicability of academic research for regulatory decision making. Whilst also set out the importance of cross-sector partnerships across industry, academia and government. As nearly a decade has passed since the initial workshop, we believe it is important to reevaluate previously identified challenges faced in applying academic research to regulatory decision-making (OBJ1), highlight challenges not previously identified (OBJ2), and assess the uptake of proposed training and skills (OBJ3). To obtain the necessary data a survey was developed and deployed online to gain understanding on the challenges early career researchers (ECRs) face in applying academic research to regulatory decision making, and the training and skills received throughout their career. In total, 52 ECRs responded to the survey. Just over a quarter of respondents (27%) consider themselves to be part of a training network, however approximately half of all respondents state that they have received training to understand the regulatory context of their research (52%); ecotoxicology methodologies or guidelines (44%); and principles of quality assurance and good laboratory practice (54%). We explore why ECRs may or may not receive training and assess the extent to which they agree that it has improved the applicability of their research for regulatory decision making. We conclude that whilst some areas of training for ECRs have developed, some areas remain a challenge. These include aspects of interdisciplinary research, cross-sector partnerships, and government or industry participation.

6.04 Methods and Tools Enabling Safe and Sustainable by Design (SSbD) Strategies

6.04.T-01 Hazard Assessment of Safe, Sustainable and Recyclable by Design Polymeric SURPASS Alternatives for Food, Building, and Transport Applications

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Plastics have become indispensable across many sectors due to their durability and versatility. However, their persistence in the environment poses severe health and ecological risks, as they degrade over centuries, fragmenting into micro- and nano-plastics (MNPs), and leaching toxic additives that pollute ecosystems and potentially disrupt human health. Currently, 70% of plastic waste in Europe is landfilled or incinerated, underscoring the need for safer and more recyclable alternatives.

The SURPASS project addresses this challenge by developing Safe, Sustainable, and Recyclable by Design (SSRbD) polymeric materials. Following the European SSbD framework, the new developed materials focus in three high-demand sectors: a) building (replacing PVC in windows frames by bio-sourced polyurethane resins); b) transport (using lightweight epoxy-vitrimer to replace metal components); and c) packaging (replacing non-recyclable films by recyclable multilayered films). These alternatives are intended to reduce environmental impact while meeting industry performance standards.

To ensure the safety of these new developed SSRbD materials, the hazard assessment herein performed included seven endpoints as their cytotoxicity and acute aquatic toxicity, and their potential to exert inflammation, oxidative stress, epigenetic modifications, genotoxicity, and endocrine disruption. All those endpoints measurements were integrated to identify the safest materials among the high priority substances identified according to the different production routes, reference route and SSbD routes: 3 hardeners, 5 different combinations of resins + flame retardant, and leachates extracted from 4 different final composites were assessed in the transport sector. In the case of building sector, 5 different bio-based polyols and 3 catalysts were studied, along with other 5 non-replaceable components. In the case of packaging sector, toxicity of the leachates of 4 multilayered films were studied.

The eco/toxicity of the materials shall be weighed and counterbalanced, allowing the selection of the materials that offer more comprehensive human safety and environmental benefits.

This study is crucial in driving safer and greener innovations in the plastics industry. By rigorously evaluating and advancing less hazardous polymers, the project sets a benchmark for sustainable design, fostering broader adoption of SSRbD principles and supporting a circular economy transition.

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6.04.T-02 Toward a better integration of Risk Assessment and Life Cycle Assessment for Safer and more Sustainable Chemicals

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The proposed presentation is a contribution toward better integration of chemical hazard data, risk assessment and life cycle assessment under the new EU framework for safe and sustainable by design (SSbD) chemicals. This framework, announced in December 2022, aims to guide the innovation process towards greener and more sustainable chemical usage by minimizing the production and use of hazardous substances. The framework consists of two main phases: the (re-)design phase and the assessment phase, which includes five steps: hazard assessment, worker exposure during production, exposure during use phase and for the environment, life-cycle assessment, and socioeconomic assessment.

The presentation highlights the importance of being consistent in using chemical hazard information generated for CLP and REACH when they are also used in life cycle assessment. The talk will present the development of a new database by the EU JRC to calculate human and aquatic toxicity impact scores using the USEtox model. This database was built using REACH registration dossiers, EFSA data for pesticides and herbicides, and data from the Pesticide Properties Database.

We explain the methodology used to curate data from various databases and the selection criteria applied to ensure the reliability of the data. A new approach based on HC20 (hazard concentration killing 20% of the exposed population) was implemented to calculate freshwater ecotoxicity effect factors. The results showed that the new approach aligns well with official toxicity rankings of substances in EU environmental risk assessment.

In conclusion, the presentation emphasizes the development of a new set of Characterization Factors (CFs) to address the limitations of the USEtox model during the Environmental Footprint pilot phase. These CFs were calculated for all available chemicals, including non-organic substances, using multiple trusted data sources. The article also discusses the open challenges in aligning toxicity-related approaches for Environmental Footprint with risk assessment to ensure the best complementarity and decision-making support for companies.

6.04.T-03 Decision Support System for SSbD of Advanced Nanomaterials

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To facilitate practical implementation of the Safe and Sustainable by Design (SSbD) approach in industrial innovation processes of advanced nanomaterials, the HARMLESS project developed a Decision Support System (DSS) that considers data availability and resources along the innovation process (<https://diamonds.tno.nl/projects/harmlesspublic>). The DSS is aligned with the EU framework and recommends the use of tools and methods, including New Approach Methodologies (NAMs) tailored to advanced (nano)materials as well as the innovation stage of the material under development. The HARMLESS DSS includes 3 flexible innovation stages: 1) the ideation and business case phase, 2) the laboratory phase and 3) the pilot phase. Designers are guided through a workflow starting with 3 tools, i.e. AMEA, WASP and ASDI. The Advanced Material Earliest Assessment (AMEA) tool consists of only 3 questions and is used for early categorization and subsequently advice on design principles and applicability of the SSbD approach. If the approach is applicable, the designer is advised to apply the second tool, named Warning flags, design Advice, Screening Priorities (WASP). WASP is based on the AMEA advice and several other existing tools as a simplified approach that requires less information. This approach consists of 12 questions to identify early warning flags on safety and sustainability and to provide design and assessment advice. To help innovators to make a decision on the most optimal SSbD version in the lab phase, another tool, named Alternative SSbD Design Inspector (ASDI) was developed. Based on the early warning flags from WASP, ASDI provides a) guidance on which descriptors to measure and b) insight into the differences between the SSbD versions within the various dimensions (safety, sustainability and performance). More detailed assessment tools, including in vivo hazard prediction methods based on physicochemical and in vitro data are suggested and available in the DSS for

the pilot phase. The DSS has been tested and improved with industrial case studies from the HARMLESS project.

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6.04.T-04 Life Cycle Based Risk and Opportunity Mapping: A Systematic Collaborative Procedure to Integrate Environmental and Health in Early Innovation

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Life cycle-based risk and opportunity mapping (LCBROM) is a qualitative screening approach to investigate potential risk and opportunities in early innovation. The goal is to push the innovation towards a more sustainable solution. This is done using a systematic MET matrix that summarizes aspects of Materials use, Energy consumption and Toxicity issues of each life cycle stage. If LCBROM is used in early innovation of materials or processes, it can reduce the risk of bottlenecks or showstoppers in a later stage of the innovation process.

The LCBROM method is an iterative process and is initiated with a framing of the assessment followed by a start-up meeting. The iterative steps of the method consists of literature reviews (allowing the LCBROM practitioner to collect information to compare the innovation to state-of-art technology) and discussions with relevant stakeholders, with continuous updates and completion of the MET matrix. Depending on whether different aspects are identified as opportunities, risks or areas requiring further investigation, this is colour-coded in the MET matrix to provide a better overview. The collaborative procedure is an important aspect of the LCBROM.

Four LCBROMs has been reported in the research programme Mistra TerraClean: i) Development of a technology to concentrate REEs from water from a discontinued mine; ii) Application of iron sulphide doped activated carbon to remove mercury from a side stream in an enrichment plant; iii) Chemical modification of PEX material to prevent unwanted bacterial growth in the distribution of drinking water; iv) Removal of CO₂ from indoor air with filters based on cellulose nano fibrils and activated carbon.

A key learning from the application of the method in the case studies is that it is essential to have a state-of-art benchmark and/or a specific case to relate the innovation to for identifying opportunities. It is, however, not a requirement for risk identification. Another learning is that a formalised stepwise approach and defined input information facilitates the execution of the method. It has also become clear that a mutual understanding of the potential and limitations of the LCBROM method is important to establish early in the assessment.

Beyond Mistra TerraClean, the method will be used in the EU funded research programme BioSustex. It also has the potential to be used in the SSbD framework outlined by JRC.

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6.04.T-05 MechoA+: One Scheme to Bind Them All

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The rise in interest for new approach methodologies (NAMs) has led to the development of a wide range of in silico approaches. Among them, KREATiS, Liverpool John Moores University (LJMU) and Unilever SEAC recently developed the MechoA+ scheme, by integrating and further refining the MechoA and Sapounidou-Firman schemes into one unique scheme. This tool is designed to help comprehend the mechanisms of toxic action related to chemical substances. It detects if the substance has a structural pattern known to be related to particular MIE(s) and for which species. Thus, it gives useful information for forecasting, controlling, and reducing the effects of chemicals on both human health and the environment as requested for SSbD assessment.

The new in silico profiler is organised by molecular initiating events (MIEs) which are the first key events in Adverse Outcome Pathways (AOPs). Each mechanism is supported by (eco)toxicological data/

knowledge from the literature. The domain of each alert of the profiler has been defined in terms of structural features responsible for the MIE and species affected. In total there are more than 150 structural alerts for various MIEs. MechoA+ alerts have been coded into iSafeRat® Desktop, providing the basic requirements for allocating a chemical to mechanistically-related QSARs. It is also being coded as a new profiler for the OECD QSAR Toolbox. In order to test the relevance of the new scheme coded in MechoA+, the SMILES strings of over 70,000 structures were screened by MechoA+ and a comparison with results from the previous MechoA and Sapounidou-Firman schemes was undertaken.

The new MechoA+ scheme shows excellent coverage, with very few compounds unassigned, and a good accuracy compared to the mechanisms inferred from literature analysis. The evaluation demonstrates the comprehensive utility of the MechoA+ scheme, while also identifying some specific areas of chemistry where mechanistic assignment is currently lacking. In particular a need was identified to create further alerts for endocrine disruption.

In most cases, the results from the new scheme complements and enhances the previous schemes with additional knowledge (e.g., species applicability, specific target hit, further key events, etc.), enhancing its usefulness for hazard assessment and understanding as well as confidence in the prediction.

6.04.P Methods and Tools Enabling Safe and Sustainable by Design (SSbD) Strategies

6.04.P-We483 Towards a Flexible, Solution-Oriented SSbD Framework in Industry: A Practical Case Study

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The European Union's Chemicals Strategy for Sustainability (CSS) emphasizes a toxic-free environment. The "Safe and Sustainable by Design" (SSbD) framework plays a critical role in assessing the safety, environmental, and socio-economic sustainability of chemicals and materials. This study presents a simplified SSbD assessment applied to two detergents used in biomanufacturing. One detergent is classified as a substance of very high concern (SVHC) due to the endocrine-disrupting properties of its degradation product, while the second is a readily biodegradable alcohol ethoxylate.

We conducted a human health and environmental risk assessment coupled with a Life Cycle Assessment (LCA), i.e., Steps 1 to 4 of the SSbD, to evaluate the detergents' safety and performance across various disposal scenarios, including incineration and wastewater treatment. The functional unit was defined as 1 kg of detergent for virus inactivation, and a cradle-to-grave assessment was performed. The overall assessment utilized several (Q)SAR and exposure assessment tools, coupled with different LCA databases, and utilized AI-based models to fill data gaps. The score drivers were significantly influenced by both the classification of the first product as SVHC and environmental Risk Characterization Ratios (RCRs) during industrial manufacturing and use. On the contrary, the second product demonstrated advantages in terms of sustainability metrics, particularly in climate change and resource use (fossil).

Challenges encountered included the identification of protective toxicological endpoints for Derived No-Effect Level (DNEL) derivation and the acquisition of tonnage information from both upstream and downstream sources. Additionally, data availability from suppliers hindered comprehensive data acquisition.

Our findings advocate for a pragmatic, stepwise SSbD application, particularly for low-volume, low-hazard chemicals, enabling a more manageable integration of safety and sustainability assessments. This case study demonstrates the potential of the SSbD framework to improve chemical safety and environmental sustainability in the Life Science sector while also tackling the complexities associated with data requirements and model integration. A step forward to assist SSbD practitioners in data filling will be the acceptance of e.g., Integrated Approaches to Testing and Assessment (IATAs), and New Approach Methodologies (NAMs) for higher-tier endpoints.

6.04.P-We484 Safe and Sustainable by Design and Circularity Operationalization Via Multiple Criteria Decision Analysis

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The Safe-and-Sustainable-by-Design (SSbD) framework of the European Commission (EC) was introduced in 2022, and its related methodological guidance as well as its application to case studies have also been recently published. The wide set of indicators that characterize the EC-SSbD framework can raise the challenge of performing trade-offs among the indicators to comprehensively evaluate the chemical or material under study. To aid this complex process, Multiple Criteria Decision Analysis

(MCDA) has been proposed as a promising methodological solution, with close to 30 studies already combining safety and sustainability. However, until now, there was no SSbD-based solution using MCDA where it is possible, at the same time, to (i) apply the framework with only one alternative, as well as with more than one, (ii) test the effect of different compensation levels on the final decision recommendation, and (iii) include circularity indicators too. To fill this gap, using an MCDA method, we propose a new composite indicator (CI) to operationalize the EC-SSbD framework. It is called CI-SSbDC and a stepwise procedure is proposed to apply our index. It includes (i) safety, life cycle sustainability and circularity assessment using five dimensions, namely hazard dimension, health dimension, environmental dimension, circular dimension, and economic dimension, and (ii) aggregation of the information to provide a score between 0.01 and 1 that characterises the overall performance of the alternatives. We tested the CI-SSbDC in three case studies, using bio-based waste streams to produce nisin. The index showed the capability of the proposed methodology to effectively deal with the underlying 26 indicators, while still providing a final comprehensive trend in the performance of the alternatives, which enables more informed decision-making. The CI-SSbDC can thus be used by process developers who are interested in comprehensively evaluating the safety, sustainability, and circularity of products at an early development stage. It suits well users that want to visualise the overall performance of products, as well as identify hotspots during their life cycle. A key novelty of this work in this area consists in the use of a MCDA method that allows to harmonize all the impact and benefit values on a common scale. In addition, it accommodates decision-makers with different preference structures, from weak to strong concepts of sustainability.

6.04.P-We485 Testing and Demonstration of the Applicability of the SSbD Framework to Develop Innovative Chemicals and Materials for replacing SVHC in High-impact Markets

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The growing safety and sustainability requirements for substances and materials, as well as current and future restrictions established in the European Regulation, have driven an escalating demand for alternatives in the chemical sector, especially for those applications where potentially hazardous substances classified in one of the ECHA hazard categories are used or developed, such as substances of concern (SoC) and substances of very high concern (SVHC). In this context, the Chemicals Strategy for Sustainability (CSS) is a key step towards achieving a zero-pollution and toxic-free environment, aiming to position the EU as a global leader in producing safe and sustainable products by design (SSbD). The holistic approach considered by EC-JRC within the SSbD framework is needed for the promotion of the assessment and substitution of hazardous substances.

In this context, the main goal of the AlChemiSSts project is to test and demonstrate the applicability of the SSbD framework to develop innovative chemicals or materials to replace SVHC in high-impact markets, notably for surfactants, plasticizers, and flame retardants in relevant value chains, including metal working fluids (MWFs), lubricants, insulation foams and paints, safety boots and wellies, battery cases and sports mats. For that, a suite of case studies including relevant actors in each value chain is being conducted to generate scientific and technical driven evidence Proof of Concept of the applicability of the SSbD framework to develop alternative substances and materials with proven safety and sustainability, able to replace existing substances of concern, as well as to identify and define specific recommendations and elements to be considered for the further refinement of the SSbD framework.

AlChemiSSts deals with the design and development of new substances and materials to be used as surfactants, flame retardants and plasticizers substitutes in real industrial applications. The project is working on the identification of the barriers to enable the implementation of the SSbD framework at all stages of the life cycle for targeted substances, materials and applications including: new approach methodologies (NAMs), data/knowledge barriers, exposure scenarios and risk assessment in processes, sustainability assessment (LCA and SEA), and definition and communication of proposed solutions and recommendations to foster and incentive the application of the SSbD framework.

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6.04.P-We486 The MAUT Approach for the Evaluation and Comparison of Functional Alternatives: A Case Study on Fluorinated Gases Used in Insulation Materials

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Although the elimination of substances of concern is the most effective measure to decrease the risk they

represent, a careful evaluation of potential alternatives is needed to prevent regrettable substitution from happening. To that end, different alternatives assessment frameworks were developed in order to identify, evaluate, compare, and select safer alternatives to a chemical of concern. However, recently it has been demonstrated that more research is still needed to ensure proper implementation of those frameworks, and efforts are especially needed to advance and incorporate flexible and practical approaches for trade-off considerations in decision-making. Multicriteria decision analysis (MCDA) methods, including the multi attribute utility theory (MAUT) approach, were suggested to face these challenges. By taking the example of fluorinated gases used as foam blowing agents in insulation materials, this study aims to propose a method based on the MAUT approach to compare functional alternatives to a chemical of concern based on their environmental impacts along their life cycle, and their technical performance. The functional substitution approach was followed to define the functions delivered by fluorinated gases in insulation materials, and the ZeroPM alternatives database was used to identify functional alternatives. Data on environmental impacts along the life cycle, and the technical performance of the identified alternatives were collected based on previous literature reviews on insulation materials. The data were complemented by a semi structured literature search when necessary. The MAUT approach was used to compare the different alternatives. Different decision-making scenarios were defined in order to illustrate the flexibility of the MAUT method for the assessment of functional alternatives. Overall, 9 alternatives to polyethene foams and extruded polystyrene foams containing fluorinated gases were identified. 7 materials could be considered as more sustainable based on their environmental impacts along their life cycle compared to the insulation foams containing the fluorinated gases. When considering technical performance in addition to the environmental impacts in decision-making, one insulation material was identified as a suitable alternative, which suggests that the use of fluorinated gases in insulation materials can be phased-out.

6.04.P-We487 Addressing Data Gaps for Safe and Sustainable by Design: A Substance-Use Dataset to inform on Functional Alternatives for PMT/vPvM Chemicals

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Persistent, mobile and toxic (PMT) as well as very persistent and very mobile (vPvM) pose a threat to drinking water quality and ecosystem health. PMT/vPvM substances do not readily degrade in the environment, can travel large distances and are difficult to remove with current water treatment technologies. PMT chemicals also exhibit toxicity to humans and biota. Hence, there is an urgent need to phase out these hazardous substances. An efficient process to identify functional, safe and sustainable by design (SSbD) alternatives is critical to achieving this. However, the scattered and rather generic nature of knowledge of the specific substance-use combinations for PMT/vPvM chemicals is a major barrier to assessing the suitability of potential alternative substances.

The research presented in this poster aims to address this challenge by manually compiling a high-quality dataset of substance-use combinations for a defined group of high-volume PMT/vPvM substances registered under REACH. Use categories, key functions and properties of each chemical and fundamental to the uses are extracted from databases, such as the substances in preparations in Nordic countries (SPIN), as well as patent literature and systematically catalogued. This allows for additional evaluation of the necessity of P, M and T properties for individual uses and informs future SSbD assessments of potential functional alternatives. This approach, targeting specifically the high-volume PMT/vPvM chemicals, allows for an in-depth assessment of all relevant substance-use combinations, enabling better evaluations of alternatives at early data-poor stages of the innovation process.

This substance-use dataset provides a critical foundation for developing and applying quantitative structure-use relationships (QSURs) to support targeted design workflows. Additionally, this dataset can facilitate the development of machine learning and artificial intelligence approaches, assisting in the knowledge collection of additional substance-use combinations. Ultimately, the compiled dataset provides a basis for allowing innovators to rapidly identify high-potential functional SSbD alternatives for further evaluation, accelerating the transition away from hazardous chemicals.

6.04.P-We488 Approaches for Safety Assessment and Sustainability of rPEX and Its Additives From Early Stages: Development of Methods and Tools to Implement the SSbD Framework

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Polyethylene is the most widely used plastic. Cross-linked PE (PEX) exhibits significantly improved properties, notably in terms of thermal stability, chemical resistance and structural integrity. As a result of

cross-linking, PEX is used in a number of applications for which PE naturally lacks required properties, or replacing potentially toxic materials, such as poly(vinyl chloride). However, due to the existence of cross-links, PEX cannot be melted and recycled/reused and is generally downcycled as a filler in other thermoplastic blends with poor mechanical properties.

To address these challenges, REDONDO project develops and applies Safe-and Sustainable-by-Design (SSbD) principles in rPEX (Reversibly PEX) and its additives design process to reduce their environmental impact and promote the design materials with these principles in early stages, by defining and assessing criteria regarding safety and sustainability of rPEX (and its additives). The results obtained from the implementation and evaluation of SSbD are being incorporated into the development of REDONDO, a digital toolbox that will support decision making for formulators and industry, guiding the development of safe and sustainable application of rPEX.

For the SSbD assessment, a tiered approach has been followed, including safety and sustainability assessment. To ensure safety, a risk assessment is carried out, involving in vitro toxicological studies based on OECD protocols, as well as an occupational exposure assessment through monitoring campaigns. To guarantee sustainability, a life cycle assessment (LCA) is implemented to identify the key hotspots for environmental improvement from the design stage in the polymer formulation process to final products. Life cycle cost (LCC) assessment has also been performed to estimate the costs of the different demonstrators to help make a more informed decision when selecting the most sustainable and viable processes and products for the scaling up.

This tier-approach is not only been carried out to assess the effects of the new polymers on humans but also plays a key role in the modification of the rPEX following SSbD strategies to obtain safer and more sustainable polymers with a reduced impact on health and the environment. The experience gained from implementing and developing strategies in the polymer sector will advance the understanding of how to move towards a greener transition in line with the Chemicals Strategy for Sustainability (CSS).

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6.04.P-We489 The SAbyNA Platform: A Guidance Tool to Support Industry in the Implementation of Safe and Sustainable by Design Concept for Nanomaterials, Processes and Nano-enabled products

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The implementation of Safe and Sustainable by Design (SSbD) strategies for materials and products is essential to achieve the Zero-Pollution Ambition stated in the EU Green Deal. Simple, cost effective and reliable methods are needed for pragmatic and flexible evaluation of safety and sustainability at early stages of innovation, especially for nanoforms and nano-enabled products, for which validated guidelines for the assessment and management of safety and sustainability are still lacking. The SAbyNA Platform has been developed by making use of existing resources that guide decisions to ensure safety and sustainability of nanoforms and nano-enabled products at the design stage of product development. Key resources from previous EU projects such as GUIDEnano (G.A. 604387) and GRACIOUS (G.A. 760840) were used. In addition, the project responded to the needs of stakeholder profiles (i.e., industry, consultants, RTOs, regulatory bodies) on how to implement SSbD through continuous stakeholder engagement. The ability of this tool to support the design of safer and/or more sustainable nano-enabled products is demonstrated through the implementation of the SAbyNA Guidance Platform in real-world case studies, such as nano-enabled 3D-printed vacuum cleaner components with antistatic properties, made with polycarbonate and single walled carbon nanotubes. The SAbyNA Platform consists of four informative modules intended to provide information to the user on the methods, models and tools suggested for exposure and hazard assessment and on Safe-by-Design interventions, and two assessments modules to conduct a screening life cycle and cost assessment, as well as a safety assessment of nanoforms and nano-enabled products. Once screening data on the additive manufacturing case study were

added, Safe-by-Design recommendations were provided, such as by reducing fiber length or rigidity or by changing the form of the NFs (e.g., using pellets/granules instead of NFs in powder form). Hazard, exposure, costs, sustainability and functionality data of the assessed case study were added in the Platform to define whether (or not) the implemented Safe-by-Design intervention was able to increase the safety of this nano-enabled product.

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6.04.P-We490 Integrated SURPASS Scoring System for Safe, Sustainable and Recyclable by Design Assessment

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EU is promoting different strategies such as the Chemicals Strategy for Sustainability and the zero-pollution ambition of the European Green Deal, to facilitate the industrial transition towards a safe, zero pollution, climate-neutral and resource-efficient production and consumption, addressing adverse effects on humans, ecosystems and biodiversity from a lifecycle perspective. In this context, the European Commission recommendation was establishing an assessment framework, promoting research and innovation for safe and sustainable by design (SSbD) chemicals and materials and the EU Joint Research Centre published different documents to guide in the implementation of the SSbD framework.

One of the main challenges of the SSbD framework concerns difficulties in implementing the steps of the assessment along the innovation process. The project SURPASS aims to deliver a web-based digital infrastructure to guide SMEs towards the development of Safe-, Sustainable-, and Recyclable-by-Design (SSRbD) polymeric materials, providing tools, methods and data to support the implementation of the assessment steps described in the framework.

Moreover, SURPASS addresses a crucial aspect of the SSbD Framework implementation, which is the integrated and overall assessment of the SSRbD steps of hazard (Step1), exposure and release (Steps 2 and 3), life-cycle and life-cycle costing (Steps 4 and 5) assessments. This was done by developing individual scoring systems for each step. Each individual scoring was based on specific score pillars that were combined to provide assessment step scores, that were then integrated to provide a final unique SSRbD score. This scoring strategy complements the assessment steps and do not aim to replace their conclusions and results. Instead, it provides a visual overview of their outcomes indicating improvements and degradations of different types of material or product performance (e.g., hazard, release, sustainability, etc.).

The final and unique SSRbD score summarizes, but at the same time hides, in one unique value all the information from the previous assessment. Therefore, it should be considered together with all the previous assessment scores and pillar scores, that allow the users to understand which aspect of the SSRbD alternative can be improved or further investigated.

This work is meant to underpin the innovation process, supporting the SSRbD approach by facilitating the decision-making process.

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6.04.P-We491 Workflow and Overall Structure of an SSbD Digital Infrastructure for SME and Polymeric Materials

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Previous and current projects indicate that many resources (data, tools, methods) are available for the risk and sustainability assessment of materials. However, these resources are not suitable for safe and sustainable by design (SSbD) implementation by small and medium enterprises (SME) as they are quite complex, require a lot of data, are time-consuming and are not always material specific. SMEs need a clear guidance on how to make their materials, products or processes safer and more sustainable. In order to support the development of SSbD polymeric materials, a web-based digital infrastructure was developed which follows the Joint Research Center SSbD framework.

The main goal of this infrastructure is to support SMEs to implement SSbD in their materials design by integrating tools and data to drive better decision-making that aligns with environmental and economic sustainability while ensuring material safety. This infrastructure consists of a four-tiered workflow to give users SSbD options based on limited data.

First, the infrastructure has an SSbD e-learning module designed to provide in-depth knowledge and practical insights into sustainable, recyclable materials and technologies across various industries.

Second, guiding principles are provided to support the design of chemicals and materials. The (re) design and SSbD leverage identification is the first phase of the two-phase approach recommended in the SSbD framework. Criteria are defined and detailed to guide the users through the (re)-design phase of the SSbD innovation. The users will then be able to reproduce the same approach as the case studies presented as examples.

Third, the assessment methodology is a step-wise tiered and iterative approach addressing hazard, exposure, environmental, economic and social impacts and providing a unique scoring strategy.

Assessment needs depend on the process maturity and three tiers are offered. This is the second phase of the SSbD framework.

Finally, the infrastructure provides access to all the project public results with a permanent access to the entire scientific outcome.

This work is developed within the framework of polymer substances and their additives (including end-of-life recycling). However, this approach can be extrapolated to other substance families.

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6.04.P-We492 Tier 1a SSbD Methodology for a Preliminary Assessment Developed within SUNRISE Project

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The policy objectives of the European Green Deal, as outlined in the Chemicals Strategy for Sustainability and the Zero Pollution Action Plan, emphasize the critical need for adopting a Safe and Sustainable by Design (SSbD) approach for chemicals and materials. To drive this transformation, the European Commission (EC) has recommended creating a European assessment framework for SSbD of chemicals and materials, based on a holistic approach developed by the EC's Joint Research Centre. To aid stakeholders, particularly within the industry, in practically implementing the EC-JRC framework, the Horizon Europe SUNRISE project is developing an Integrated Impact Assessment Framework (IIAF). This framework aims to assess the health, environmental, social, and economic impacts of products enabled by advanced materials (AdMa). The IIAF, aligned with the EC-JRC SSbD framework, follows a 3-tiered approach, with each tier offering an integrated methodology supported by a dedicated toolbox for assessing various impacts. These tiers target different user groups at various stages of the innovation process, with the first tier divided into two sub-tiers: Tier 1a and Tier 1b. This presentation focuses on the development of Tier 1a, a rapid screening method based on a 20-question questionnaire designed to assess safety and sustainability aspects in the early design stage. To develop Tier 1a, a thorough review of existing approaches, frameworks, and questionnaires was conducted to assess safety, sustainability, functionality, and regulatory aspects. The aim was to identify commonalities, especially the frequently repeated and important questions. Subsequently, a preliminary set of questions was created in collaboration with key stakeholders from industry, regulatory bodies, policy-making, and academia. A working group of experts in SSbD frameworks, nanomaterials risk assessment from various EU projects, and environmental and socio-economic sustainability was formed. This group meets periodically to design the questionnaire and determine the most relevant questions.

6.04.P-We493 Safe and Sustainable Bio-Design of Polymers for a Circular Economy

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The transition toward biobased polymers derived from second-generation (2G) feedstocks, such as agri-food waste and biomass, presents a critical opportunity to enhance sustainability and reduce dependency on fossil-based materials without affecting timber, food and other sectors. To ensure these materials meet stringent safety and sustainability criteria, the Safe and Sustainable by Design (SSbD) framework seems to be an exceptional tool to be applied. Prioritizing safety and sustainability throughout the entire life cycle of bio-based chemicals, ingredients and materials represents a paradigm shift in improving not only their sustainability profile, but other well recognized challenges in bio-based sector: raw material availability, high production costs, lack of infrastructure, complex regulations, consumer acceptance, competition with fossil-based products and scalability issues.

The present work, developed within the framework of the POLYMERS-5B project, details how all these kind of materials, including polyesters, polyamides, polyphenols, and poly-furans, are designed following SSbD metrics to mimic the properties of fossil-based polymers, such as PET and PUs, while delivering improved biodegradability and circularity and addresses all these challenges. By leveraging biocatalysis and green chemistry, these materials achieve high functionality and performance, making them suitable for applications in textiles, automotive, and furniture.

A detailed weighting system to determine score of SSbD following all these statements as well as safety and sustainability considerations are embedded in early design process. Thanks to that, innovation can advance for the development of bio-based materials that support the circular economy, providing environmental benefits while meeting market and performance demands. System allows to integrate ecodesign principles, lifecycle thinking and the reduction of chemical toxicity and risks for human health and the environment, ensuring that these solutions enhance safety and sustainability without compromising other dimensions, such as performance or economic viability. This iterative and quantitative approach, applied from the earliest stages of innovation is crucial for addressing global challenges like resource efficiency, carbon reduction, sustainability and minimizing chemical hazards.

6.04.P-We494 An Overview of the Methodology and Implementation of the Step 1 of the EC SSbD Framework for the Development of Safe and Sustainable by Design Graphene/Mxenes Hybrids

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The SAFARI project advances the development of MXenes, 2D materials derived from MAX phases, known for their high electrical conductivity, mechanical strength, and chemical stability. These qualities make MXenes promising for industrial applications, including conductive inks, biosensors, and electromagnetic shielding paints. However, current production methods often rely on hazardous chemicals, raising human and environmental safety concerns. The project adopts Safe and Sustainable-by-Design (SSbD) principles, utilizing REACH-compliant chemicals and conducting thorough safety and sustainability assessments for MXenes and hybrid MXenes with graphene.

The implementation of Step 1 of the SSbD framework focuses on analyzing the intrinsic hazards of materials. A hazard pre-assessment classified materials using data from the European Chemicals Agency (ECHA) database, including classifications of Substances of Very High Concern (SVHC) and REACH-restricted substances.

A decision tree was developed to guide subsequent actions based on the registration status of materials. For unregistered materials, in vitro toxicity tests will assess human and environmental hazards. For registered materials, hazard evaluations occur in three stages: Stage 1 utilizes ECHA CL inventory data; Stage 2 addresses data gaps with QSAR models like VEGA, QSAR Toolbox, and EPI Suite, as well as prediction tools and literature reviews; Stage 3 identifies hazard classes not applicable under European regulations and those lacking sufficient data.

Challenges emerged, such as limited QSAR model capabilities and low reliability in some cases. Additionally, newer toxicological categories like Endocrine Disruption (ED), Persistent Bioaccumulative and Toxic (PBT), and Persistent Mobile and Toxic (PMT) lack robust data, complicating accurate classification.

The decision support tree and strategies for addressing data gaps enable more comprehensive Step 1 implementation within the SSbD framework. After completing hazard evaluations, levels were assigned, and a final score determined material suitability. This score identifies substances requiring replacement, those necessitating safety measures, and those safe for use. Alongside subsequent SSbD steps, it informs material development, exposure assessments, and sustainable scaling of production processes, reducing hazards, risks, and environmental impacts.

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6.04.P-We495 Probabilistic Multi-perspective Application Selection for Safe and Sustainable-by-Design: A Case Study on Biochar

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Here, we propose the Probabilistic Multi-perspective Application Selection (pMPAS) to identify Safe and Sustainable-by-Design (SSbD) applications for novel materials. Although not a novel material, biochar has been selected as the subject of this pMPAS case study because its growing prominence as Negative Emissions Technology (NET) is also fostering novel applications. Novel biochar applications aim for extended carbon storage (particularly for low-quality biochars that degrade faster) by prolonging biochar's use phase, delaying its environmental release, and consequent degradation. Nevertheless, an SSbD assessment for novel biochar applications is necessary to understand and balance trade-offs between NET and toxicity concerns (from heavy metals, polycyclic aromatic hydrocarbons, etc.). Therefore, this pMPAS case study stochastically scores the functionality, (human and environmental) safety, (environmental) sustainability, and economics of 15 novel biochar for an SSbD ranking wherein Animal Feed, Passive Cooling, and Concrete Aggregates rank the highest, whereas Reductants, Plastic Additives, and Carbon Nanomaterials rank the lowest.

Keywords: Biochar, Negative Emissions Technology (NET), Safe and Sustainable-by-Design (SSbD), Probabilistic Multi-perspective Application Selection (pMPAS)

6.04.P-We496 Streamlining Safe and Sustainable by Design Practices: A Knowledge-integrated Toolbox and Workflow Approach supporting Innovative and Safer Product Design

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This presentation will emphasise the incorporation of Safe and Sustainable by Design (SSbD) principles into the development of new products. Traditional product safety assessment processes frequently rely on lengthy animal testing and may fail to accurately predict and manage impacts on human and environmental health and their interactions, highlighting the urgent need for a move toward more ethical and comprehensive assessment methods. Within our framework, New Approach Methods (NAMs), such as high-throughput screening, in silico and in vitro methods, and omics technologies, are integrated into tiered strategies such as Integrated Approaches to Testing and Assessment (IATA), Next Generation Risk Assessment (NGRA), and SSbD to incorporate safety and sustainability into product design from the start of the innovation and development process.

Our developed toolbox includes a variety of tools, guidance manuals, databases created using harmonised data templates for collected data and data generated and managed following FAIR principles, incorporated into a workflow system implementing SSbD tasks and decisions. The toolbox is designed to assist product designers, innovators and risk assessors by incorporating safety and sustainability considerations into the product and process lifecycles from early innovation stages. We will specifically detail how we are creating a workflow system that allows users to use this toolbox effectively, hence improving their ability to integrate the concepts and calculations into daily operations and decisions.

Through application to a variety of industrial case studies, we demonstrate how this approach simplifies the application of the toolbox in real-world scenarios, guiding early design decisions and ensuring that products are both safe and environmentally sustainable.

We will explain with detailed reference examples how our solutions are enabling innovative product development combined with safety assessment, shifting the work process from a reactive to a proactive practice that anticipates new regulatory standards and aligns with industrial and consumer expectations for safer, higher quality, and more sustainable products.

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6.04.P-We497 Comprehensive Hazard Assessment of Novel Biobased Coatings Under the SSbD Framework - The TORNADO Project

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The human health and environmental risks of poly- and perfluorinated alkyl substances (PFAS) has led to bans and restrictions on their production and use and highlighted the need for safer and more sustainable alternatives to be used in coating applications. Aligning with the EU Green Deal and Chemicals Strategy for Sustainability strategies towards a circular economy and a toxic-free environment the TORNADO project aims to develop new organic and hybrid PFAS-free coatings that are safe and deliver environmental, societal, and/or economical value through their applications in packaging, textile, and kitchenware. To ensure that those coatings present indeed a safer and more sustainable alternative to PFAS-based ones, TORNADO is set to closely follow the criteria defined under the EU Safe and Sustainable by Design (SSbD) framework. A safety assessment of the TORNADO chemicals and materials is dictated under the SSbD framework which among others requires a comprehensive hazard assessment of the intrinsic properties of the chemical or material in order to understand their hazard potential before further assessing the safety during use. Using a combination of literature data, in silico QSAR (Quantitative Structure-Activity Relationship) and machine learning based tools and read-across methods the human health and environmental hazard profiles of TORNADO candidate coatings are generated. The hazard endpoints assessed include environmental fate properties (i.e., P, B or M criteria) as well as relevant toxicological endpoints such as mutagenicity, carcinogenicity, developmental and reproductive toxicity, endocrine disruption, skin sensitization and aquatic toxicity among others. As part of the assessment the degradation/transformation products of the main building blocks of the TORNADO coatings are predicted using in silico transformation pathway prediction tools and the hazard profiles of major abiotic and biotic degradation products are considered. An automated workflow is developed and applied in the form of a hazard screening tool that involves the following steps (1) screening over selected regulatory and other hazard information databases as well as the execution of in silico prediction models to collect both experimental and in silico hazard data, (2) generation of a chemical/material hazard profile as a straightforward heat map, and (3) identification of data gaps and/or data uncertainties.

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6.04.P-We498 The PARC Safe and Sustainable by Design toolbox: An Integrative Toolbox for the Operationalization of SSbD

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In 2022, the European Commission (EC) introduced the Safe and Sustainable by Design (SSbD) framework, a voluntary premarket approach that promotes chemical innovation through the integration of safety and sustainability early in the design phase. The SSbD framework, as described by the EC Joint Research Centre (JRC), comprises two phases, the (re)design and assessment phases, respectively. One of the objectives of the European Partnership for the Assessment of Risks from Chemicals (PARC) is the development of the PARC SSbD toolbox for the operationalization of the EC SSbD framework. The PARC SSbD toolbox is designed to serve as a comprehensive and integrative toolbox for professionals, researchers and regulators that will provide all relevant methods, tools and data for SSbD. The alpha version of the PARC SSbD toolbox is nearing completion and is structured around an enhanced methodological framework, that integrates advanced methods for hazard, exposure, risk, and sustainability assessment. A comprehensive overview and review of available tools that can support SSbD was conducted. The tools were structured and mapped in accordance with the five stages of innovation (stage-gate model) and the five SSbD steps. The launch of version 0.1 the testing version of the SSbD toolbox introduced two new interfaces designed to enhance the assessment and decision-making process,

accordingly: the SSbD wizard and the SSbD Scoring and Evaluation tool. These two elements are further refined during the development of the alpha version of the toolbox. Two tools have been successfully integrated VEGA and INTEGRA and additional tools will be incorporated into the platform. The PARC SSbD toolbox development follows a concurrent engineering and iterative approach. As it progresses towards its first official version, the development of a more informative decision support system that will facilitate the design and assessment of new and existing substances is structured.

6.05.A Implementing Holistic SSbD Approaches to Chemicals and Materials: What Do Academia, Industry, Regulators and Policymakers Propose?

6.05.A.T-01 Enhancing the Communication in the Value Chain for the Safe and Sustainable by Design Framework Implementation

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"Key for the green industrial transition is the SSbD framework developed by the JRC to ensure the placement on the market of chemicals, materials and products that are safe and sustainable by design (SSbD).

The framework puts together safety, environmental sustainability and socio-economic dimensions for the first time. In addition, it requires consideration of the safety and sustainability throughout the entire life cycle of chemicals, materials and products, starting this assessment from the beginning of the R&D, shifting away from the current practice. Thus, the framework goes beyond current legislation and represents a paradigm shift in the way that innovation is done.

In a first step towards improving the relevance, reliability and operability of the SSbD framework in R&I activities, envisioned by the European Commission (EC) Recommendation, the JRC has published the first methodological guidance reflecting the experience gained since the publication of the Framework in relevant works like:

- a) The application of the Framework to three case studies: i) Non phthalate plasticisers in Food Contact materials, ii) Enzymes in the scouring process of textiles and iii) Halogen free flame retardants in Information and communication technologies.
- b) The 1st edition of the SSbD Bootcamp organized and hosted by the JRC.
- c) The first feedback collection phase of the 2-year testing period proposed by the European Commission's (EC) Recommendation establishing a European assessment framework for safe and sustainable by design (SSbD) chemicals and materials [5].

Among the novelties introduced by the guidance, the scoping analysis highlights the importance of life cycle engagement in the SSbD Framework implementation that will be the focus of this presentation. The presentation enlarge on the broader meaning of the life cycle perspective and the importance of expert engagement (with a particular focus on the value chain experts) for the successful implementation of the Framework.

6.05.A.T-02 Stakeholder Engagement for Advancing Socio-economic Assessment in Safe and Sustainable by Design

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Over the past few years, it has become increasingly clear that concepts such as Safe and Sustainable by Design (SSbD) are an important part of the early stages of innovation and product development. However, there is still a lack of expertise on what needs to be considered in a thorough assessment as well as access to reliable databases for the implementation of SSbD. In particular, further developments of the SSbD approach within the fifth and final step of the framework, the socio-economic assessment are required. In early innovation stages, this is still challenging as final material compositions and actors in the value chain are often unknown. In addition, there is a lack of knowledge and strategies on how to fill this gap to propose a holistic and implementable SSbD approach. Hence, the focus of the present work is to investigate the possibilities to assess potential social impacts with a focus on two different value chains in the pharmaceutical industry (i.e., a COVID-19 vaccine available on the market and a nanoemulsion in early stages of development). Within this study, a multi-method approach is established to investigate 1) which methods are available to quantify social risks/impacts in the context of SSbD, 2) which stakeholders need to be considered and which topics should be included for the assessment of the pharmaceutical products and 3) which topics can be related to technology or product-related design parameters. Besides reviewing relevant literature and desktop research, the stakeholder consultation process is a three-step

approach: (1) expert interviews to explore stakeholder groups and relevant social topics to be considered; (2) a standardized survey to prioritize topics among stakeholders and experts in the industry; and a focus group workshop with people developing pharmaceuticals to identify technology relevant social topics. The obtained results are a list of social topics which can be related to technology type and feature including methods and indicators for quantification that should be considered in step 5 of the SSbD when assessing product systems in the pharmaceutical industry. In conclusion, the present study underscores the need for streamlined tools for early-stage assessments and collaborative approaches to stakeholder engagement to fully integrate social considerations into SSbD practices in the pharmaceutical industry.

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6.05.A.T-03 Combining Policy and Industry Perspectives for building a Safe and Sustainable by Design Scoping Approach

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Safe and Sustainable by Design (SSbD) has a broad vision and requires technical expertise in safety and sustainability assessment & design. Policy & industry are grappling with a practical operationalization of SSbD. EC's SSbD framework is currently being systematically refined with stakeholder feedback & case study application. Safety & sustainability considerations in chemical companies are included as part of Portfolio Sustainability Assessment, and often included in stage gates. SSbD operationalisation requires development of content and process aspects of a scoping approach. Scoping involves contextualization of SSbD in early innovation, prioritizing parameters, linking assessment to (re)design & providing a decision logic to support SSbD results interpretation. PARC 8.1 conducted two activities: a) comprehensive tool testing case study on Bisphenol A, b) group interviews of companies on how they currently conduct safety and sustainability assessment during innovation. A scoping approach is being developed by combining the top down perspective of policy and industry, with the bottom up perspective from tool application. We describe how a scoping approach is being developed in PARC 8.1: a) How does industry currently apply safety and sustainability in their innovation process? b) What are the similarities and differences between the EC and CEFIC approaches? c) What aspects of the EC and CEFIC guidance does the BPA case study currently cover? d) What are recommendations for further development of the scoping approach? The key findings of this investigation for developing a scoping approach include: a) While innovation within chemical companies is diverse, depending on the sector and starting point, we find that the SSbD system is operationalized through a multidisciplinary team including SSbD topic experts along with an innovation manager. b) The early stages of innovation are particularly influenced by expert judgement and other qualitative approaches, c) The comparison of the EU and CEFIC guidance showed that the two frameworks address different levels of SSbD, and thus differ in their focus on SSbD assessment/redesign content and process. Some assessed criteria include differences in focus on innovation, SSbD assessment level, assessment unit of analysis, assessment approach, (re)design approach, and trade-off approach. Recommendations for the development of a practical scoping approach to support operationalization of SSbD will be discussed.

6.05.A.T-04 Value Creation and Tiering: Incentives of SSbD Integration Into Industrial Innovation Projects With LCA and Measured Safety Screenings

Wendel Wohlleben, BASF SE, Germany

The sustainability transformation is not reached by endorsement alone, but by a myriad of innovations. Qualitative considerations of SSbD principles are common in industry, but quantitative approaches to early screenings of the different SSbD dimensions including hazard, occupational safety, environmental and consumer safety, environmental sustainability, cost, performance, and environmental benefits are rare. In the present contribution, we focus on incentives, and develop scenarios in which SSbD creates an added

value also in the well-established economic measures of an R&D pipeline. We employ the established measure of the Expected Commercial Value (ECV), which is a common basis for the investment decision at every gate. It depends on a business case with projected costs for the technological development, and costs for market launch. In our SSbD scenarios, we assume that the consideration of tradeoffs (not favoring simply the best performing material, but the one with the best balance of all SSbD dimensions) has an impact on the probability of technological success. We also postulate that under the big societal trend towards the sustainability transformation, the value chain will increasingly see a value in more formalized, more standardized, more quantitative SSbD approaches, thus impacting the probability of commercial success. In one scenario for a project aiming to develop a high-value innovative product, one can demonstrate that the application of full SSbD at early R&D stages removes the economic incentive to fund such a project (Figure 2). It becomes evident that it is essential which intensity of SSbD work at which time is planned, because there is a high probability that the project will never come to that stage because of the overall evaluation of all SSbD dimensions, including technical feasibility, cost and performance, at each gate. However, tiering with a selection of NAMs nearly turns the tide, and the profitability is restored if the value chain sees a value in the implementation of more formalized SSbD practices. In summary, if the SSbD efforts are adapted to the scenario, are systematically tiered, starting from scoping and simplified comparative assessment, and if the market adoption depends on a justified balance of all SSbD dimensions, then there is an economic incentive, and SSbD pays off.

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6.05.P-Tu446 A Multi-actor Approach to Facilitate the SSbD Implementation in SURFs UP Bio-based Value Chains

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Currently only 4% of the surfactant market share consists of compounds that are 100% based on biomass (fully biobased surfactants), while there is an urgent need for non-hazardous Safe and Sustainable by Design (SSbD) biosurfactants (BSs). The biosurfactant market is expected to grow at a CAGR (Compound Annual Growth Rate) of between 5.2 and 10.5% between 2022 and 2028. However, the diversity of the currently available safe and sustainable biosurfactants is not at all sufficient to meet the market needs: diversity is key. SURFs UP proposes a (integrated) combination of biological & green chemical production routes from (waste) biomass second generation (2G) feedstocks and following SSbD criteria which will allow a dramatic transformation of the surfactant market by offering the broad diversity of safe and sustainable alternatives at a reasonable cost that the market is looking for. SURFs UP will bridge this gap by demonstrating optimized and cost-efficient processes for 9 SSbD biosurfactant products that will be formulated in prototypes in home- and personal care and agrochemical applications. These ingredients will be fully characterized and evaluated according to the EC SSbD Framework criteria. Finally, the production of SSbD BSs will be scaled up and used to demonstrate prototype formulations in home care, personal care, and biopesticide applications. SURFs UP project activities implement a multi-actor approach according to the quadruple helix innovation model, where science, policy, industry, and society are involved in the technology development and innovation process. This ensures that all the relevant actors and end-users in the bio-based value chain are appropriately involved. In SURFs UP a decision-making process will be implemented (e.g., stage to gate) along the supply chain to promote the integration of the SSbD principles in all stages of product development, use and end-of-life. We ensure an active and iterative flow of information from the start of the project, with all the actors involved in the products development, through meetings and workshops. Important information will be produced throughout the project which will be analyzed in an integrated way.

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6.05.P-Tu455 How Can Innovation Management Support SSbD?

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The Safe and Sustainable by Design (SSbD) framework is a transformative approach to innovation, embedding safety and sustainability into the design of chemicals and materials. Addressing the "triple crisis" of climate change, biodiversity loss, and pollution, it aligns innovation with societal, safety and environmental goals. However, operationalizing SSbD principles demands rethinking traditional

innovation processes to integrate safety and sustainability alongside economic and functional performance, introducing new complexities and uncertainties.

Key challenges include managing trade-offs across safety, environmental, social, and economic dimensions, evaluating long-term impacts, and addressing stakeholder concerns from the design phase onward. While managing uncertainty is inherent in innovation, SSbD introduces novel uncertainties related to sustainability metrics, regulatory requirements requiring systematic and adaptive solutions.

Structured innovation management systems (IMS), such as those outlined in the ISO 56000 can guide alignment with organizational objectives through leadership commitment, strategic direction, operational planning, and continuous improvement. By integrating SSbD principles into an IMS, organizations could potentially foster a culture of innovation that prioritizes safety and sustainability.

The ISO 56000 series outlines eight principles that could support SSbD, for example realizing value, future-focused leadership, adaptability, and managing uncertainty. However, IMS frameworks can be complex, particularly for SMEs. To address this, the study will focus on a simplified model, 4F (Capability for Renewal). 4F focuses on monitoring external trends, managing internal resources, goals with purpose, and the innovation process.

By exploring synergies between ISO 56000 and SSbD, this work offers a first view into practical strategies for embedding safety and sustainability into innovation processes.

6.05.P-Tu462 Bringing Safe-and-Sustainable-by-Design to the Public – Exploring Stakeholders' Concerns and Needs Towards Nanotechnologies

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Nanomaterials are characterised by their great potential in research and industry, but at the same time are still relatively little known to the public. Even though many application areas of nanomaterials, including cosmetics, medicine and electronics, are advancing to provide a more Safe-and-Sustainable-by-Design (SSbD) approach, public perceptions towards nanotechnology are sceptical. Within this study, a multi-method approach was used in order to examine relevant factors on public and stakeholders perceptions. Firstly, a multi-level media analysis in Germany observed the societal discourse on nanomaterials, different actor groups and factors of public acceptance. This analysis included data from newspaper articles (N=272), semi-scientific magazine articles (N=99) and on the social media level user comments from thirteen YouTube videos on nanotechnology advancements. Following, expert interviews and a pedestrian survey in Germany (N=34) were conducted to prepare an expert workshop (N=20) to discuss the potential and challenges of public trust towards nanomaterials in sunscreens. In addition, a survey along stakeholders in nanotechnologies was conducted.

Findings from the media analysis showed that the reporting of technology development was predominantly descriptive and did not discuss the social implications for the public. There was an ambiguity concerning the health and ecological risks of nanomaterials in the articles, which portrays the scientific uncertainty of this technology. Furthermore, social media users were opinionated towards nanotechnology, ranging from sceptical views of potential misuse to hopeful and curious outlooks on the future of applications. There tends to be disappointment in not receiving the promised advancements in nanoscience, shaped by its intangible nature and existing associations of the technology. Thus, there is a need for continued efforts to enhance public engagement by increasing and diversifying the range of topics in news reports and creating outreach activities from social media channels to disseminate information. Based on the results and under the motto Bringing SSbD to the public, information and communication materials such as factsheets and a nano-quiz were developed and distributed, thereby gaining important experience in how people can be reached and addressed on this complex topic through science communication.

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6.05.B Implementing Holistic SSbD Approaches to Chemicals and Materials: What Do Academia, Industry, Regulators and Policymakers Propose?

6.05.B.T-01 Redefining Drug Innovation: How to Integrate the Safe and Sustainable by Design (SSbD) framework to the Pharmaceuticals and Nanomedicines Sector

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The pharmaceutical industry plays a vital role in global health but faces growing scrutiny over environmental risks posed by active pharmaceutical ingredients (APIs) and their metabolites. These compounds, often entering the environment through excretion or manufacturing, persist and impact ecosystems. Examples include the feminization of fish caused by 17 β -ethinylestradiol. The detection of over 156 APIs in European waters, with concentrations up to 10 μ g/L, highlights the need for sustainable solutions.

The Safe and Sustainable by Design (SSbD) framework, part of the Chemical Strategy for Sustainability, aims to integrate safety and sustainability into material design from the earliest stages. However, adapting SSbD to pharmaceuticals requires addressing challenges such as strict regulatory requirements, long R&D timelines, and emerging technologies like nanomedicines.

This study examines how SSbD principles can fit into the drug development process. Key phases such as Drug Discovery, Lead Optimization, Pre-clinical Studies, and Clinical Trials, were analyzed to identify integration points without disrupting functionality. Existing data on toxicology and pharmacokinetics were found to support SSbD, reducing additional testing and costs. Three scenarios were evaluated: Simplified SSbD, starting early but increasing complexity; Intermediate SSbD, introduced during Lead Optimization for balance and re-design opportunities; and Complete SSbD, applied late with limited flexibility. The Intermediate scenario was deemed the most practical, leveraging existing data while minimizing costs.

Cost analyses showed modest increases: \$1.9 million (0.033% of the phase budget) for hazard assessments during Lead Optimization and \$10,000-\$30,000 per compound (0.22%) for Life Cycle Assessments in Pre-clinical Studies. To enhance sustainability evaluations, the EA-DALY (Environmental Assessment Disability-Adjusted Life Year) metric was proposed, balancing health benefits with environmental impacts. The handprinting approach complements this by highlighting positive lifecycle contributions, such as reduced environmental burdens.

Nanomedicines introduce unique challenges due to nanoparticle complexity, requiring tailored hazard assessments and alignment with advanced materials guidelines. By adapting SSbD, the pharmaceutical sector can address these challenges while supporting innovation and sustainability.

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6.05.B.T-02 Enabling Safe and Sustainable Innovation: Transparent Decision Support

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Safe and Sustainable by Design (SSbD) provides an opportunity to proactively design innovative products that not only provide solutions to societal challenges, such as the energy transition, but also ensure no harm is caused to humans or the environment. Achieving this delicate balance between performance, health, safety, sustainability, and costs necessitates an integrated approach during all phases of product innovation.

We present a transparent and tiered decision support approach for companies to implement SSbD for their products or portfolio, and for regulators to transparently visualize the impact of substitution. The engagement of relevant stakeholders is key in the approach. In the first step, design criteria for performance, health, safety, sustainability and costs are selected based on stakeholder input, including company management, investors, regulators and local residents. The second step is a screening-level assessment of the potential human health, environmental and social hotspots in the life cycle of the chemicals, materials, and products under consideration. The third step is a transparent visualization of the performance, health, safety, and sustainability profile of the selected design, complete with uncertainty indicators. The decision support approach identifies specific aspects that demand additional attention or

action. The fourth step is to bring the relevant people together to discuss trade-offs between SSbD aspects and make a decision on the next step of the product/chemical design. In case information is lacking to allow decision making, the fifth step is to gather additional information by for example conducting measurements, which will feed again into the visualization to aid decision making. These steps are iteratively repeated at successive development stages of the product innovation.

To demonstrate the transparent SSbD decision support, several case studies have been conducted. The output shows that the SSbD decision support empowers industries to test scenarios and effectively balance risks and benefits along their design process, taking stakeholder needs into account. Moreover, the approach supports regulators to transparently visualize the impact of substitution of substances of concern thus avoiding regrettable substitution and contributing to the creation of a healthier and more sustainable society.

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6.05.B.T-03 Benchmarking the “Safety and Sustainability by Design” (SSbD) Culture

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In the domain of nanotechnologies, safety and sustainability can be anticipated and actuated from the earliest stages of development of new particles and advanced materials, nano-enabled products, and Research & Innovation activities. Throughout the early 2020s, a strong and accelerating movement is observed in academia, government, and industry towards definition, operationalization, and application of such Safe and Sustainable by Design (SSbD) concepts and strategies.

This mindful movement has a sociocultural dimension, elaborating its own practices, vocabulary, relationships among stakeholders, beliefs, values, and more. Study of SSbD's emerging sociocultural profile in this seminal period of rapid development can capture areas of controversy and ideas in transition, and furnish an interesting benchmark. In 2023, the Responsible Research and Innovation (RRI) team of the Horizon 2020 SAByNA project therefore performed a large anonymous survey (N=172) of how the nanotech and advanced materials communities conceive of SSbD. A pilot study (n=161) from 2021 enabled comparisons and trend analysis.

Empirical social psychology methods and concepts were employed to benchmark so-called "social representations" of SSbD. Expert stakeholders were invited to share their implicit definitions, agreement or disagreement on selected facets of achieving nanosafety and sustainability. Analysis shows increasing complexity of SSbD concepts within the community. Preventive safety strategies emerge as a frame for considering materials and processes, and as a driving force to achieve One Health -type outcomes. Environmental aspects of sustainability are foremost in mind, but a strong desire to develop socio-economic aspects is clear. An SSbD community culture emerges, characterized by the powerful and consensual conviction that SSbD will improve environmental health and occupational safety. Participants recognize that SSbD induces a new mentality and are highly engaged. Areas of dissensus were identified as well. While respondents agree that SSbD will not hinder innovation, views on operational challenges, including cost, are more scattered. We identify these less settled attitudes as warranting further discussion and debate.

Such survey results, and moreover repeat measures in coming years, can support interdisciplinary, cross-sector stakeholder dialogue, consolidate the community, and facilitate access to improved SSbD guidance.

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6.05.B.T-04 An Auto-diagnostic SSbD Tool to Facilitate the Integration of Safe and Sustainable by Design Approach in the Innovative Stage

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Since the publication of the draft Safe and Sustainable by Design (SSbD) framework by the Joint Research Center, the European Commission has initiated several actions to support the evaluation and future deployment of this approach. However, the SSbD assessment remains a complex and resource-intensive

process. This study proposes an initial, simplified SSbD assessment to minimize data requirements, reduce time investment, and lower the level of specialized expertise necessary, aiming to make SSbD more accessible and efficient while preserving its core objectives.

A three-tiered approach is introduced, with Tier 1 focusing on a simplified evaluation applicable to all considered alternatives. This tier is designed to facilitate early-stage integration of the SSbD approach. A generic questionnaire was developed to enable easy comparison between the reference and alternatives across four SSbD dimensions: safety, environment, economic, and social. The Tier 1 assessment serves as an auto-diagnostic tool, evaluating each SSbD dimension through a set of up to 10 generic questions. The user selects a score (0 for "No" or "I don't know", 5 for "Partly", and 10 for "Yes") for each question, and results are aggregated to provide a percentage score for each dimension, which is then weighted to yield an overall score. This evaluation highlights gaps and provides a foundation for developing an improvement plan and guiding higher-tier SSbD assessments.

The Tier 1 assessment has been applied to molecules and final products (flame retardants, plasticizers, surfactants) as well as processes (hydrogen production), demonstrating its versatility as a "quick-and-easy" pre-assessment tool.

The primary advantage of this Tier 1 auto-diagnostic tool is its ability to enhance the agility and adaptability of research and innovation (R&I) strategies. While it offers a first-level identification of the SSbD status of an alternative with minimal data, it also enables the identification of key gaps and outlines necessary next steps. In the comprehensive, multi-tiered SSbD approach, the generic questions from Tier 1 will be refined and consolidated in higher tiers, where more detailed, expert-driven assessments (Tier 2 and Tier 3) will involve a deeper focus on each SSbD dimension, including the participation of R&I experts.

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6.05.P-Tu442 Screening Sustainability Assessment of Innovative Bio-Based Solution for Art Restoration

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Within the HEU GREENART project, an assessment approach was developed to evaluate the safety and sustainability of innovative solutions for art restoration, including protective coatings, foams and packaging materials, consolidants, gels and cleaning fluids, and green tech sensors. This approach, developed taking in consideration the Safe and Sustainable by Design (SSbD) Framework and the Nanorestart Framework, is structured into three iterative stages. Stage 1 involves the Hazard Assessment of innovative ingredients and formulations. Stage 2 includes a Screening Sustainability Assessment. Stage 3 focuses on assessing the sustainability performance of the most promising solutions identified in the earlier stages, through Life Cycle Assessment (LCA). This study focuses on the stage 2 of the GREENART approach: the Screening Sustainability Assessment, developed during the second year of the project and already used to guide the decision-making process. This assessment is conducted through a questionnaire structured around three main aspects: Functionality, Safety and Sustainability. The three aspects are assessed across the entire life cycle of GREENART's innovative products and benchmarks. Each aspect is assessed through specific criteria, providing measurable indicators of sustainability performance. The criteria help in determining the significance and impact of each aspect within the life cycle stages. Finally, the assessment incorporates targeted questions designed to evaluate compliance with the established criteria. Each question has five possible answers, representing the level of satisfaction with a specific product solution, ranging from "excellent" to "bad". These scores are then averaged to visualize the overall results across the different life cycle stages and sustainability aspects.

The safety aspect is assessed by combining the Hazard Classification from Stage 1 of the GREENART approach with an Exposure Classification, inspired by the ProScale method, within the GREENART Control Banding Matrix.

The results of the Screening Sustainability Assessment will be presented for one case study, selected from GREENART's innovative products and related benchmarks.

This approach aligns with the SSbD framework, ensuring compliance with evolving European and

international chemical safety regulations while continuously improving the safety and sustainability profile of these products.

6.05.P-Tu447 The SUNRISE SSbD Integrated Impact Assessment Framework

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As part of the European Green Deal, the Chemicals Strategy for Sustainability and the Zero Pollution Action Plan highlight the critical need for a Safe and Sustainable by Design (SSbD) approach to chemicals and materials. To drive this transformation, the European Commission (EC) has recommended the establishment of a European assessment framework for SSbD of chemicals and materials, based on a holistic approach developed by the EC's Joint Research Centre. To facilitate the practical adoption of the EC-JRC framework by stakeholders, particularly within the industry, the Horizon Europe SUNRISE project has been funded to develop integrated methodologies for assessing the health, environmental, social, and economic impacts of products enabled by advanced materials (AdMa). The SUNRISE project combines the bottom-up development of methods and tools for assessment of health, environmental, social and economic impacts with their top-down integration in an overarching Integrated Impact Assessment Framework (IIAF). This framework is designed to support SSbD decision-making along supply chains and lifecycle stages of AdMa and their products. The process of developing and testing the IIAF in cocreation with the stakeholders within a trusted real-world environment will improve our understanding of stakeholders needs, perceptions and relevant SSbD aspects to be considered, along with potential safety and sustainability trade-offs. This new knowledge will be transferred to regulators and policy makers at EU and national level to support them in the implementation of SSbD-related policies for chemicals and materials. The IIAF is composed of 4 assessment Tiers, fully aligned to the 5 steps of the EC-JRC SSbD framework, where each tier corresponding to an integrated methodology (supported by a toolbox) for health, environmental, social and economic impact assessment targeting different groups of users at different stages of the innovation process and requiring a different level of data and expertise. The implementation of the IIAF will ensure better regulatory compliance for AdMa based products and can have an impact on shortening the time of new materials/products to reach the market, thereby supporting the Green Deal policy objectives for the transformation of the EU into a modern, resource-efficient and competitive economy.

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6.05.P-Tu458 Methods and Tools to Assess Exposure and Risk for Steps 2-3 of the SSbD Framework for Advanced Materials

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The European Commission's Safe and Sustainable by Design (SSbD) framework represents a pivotal initiative to integrate safety, environmental sustainability, and societal benefits into the design of materials and chemicals. Advanced Materials (AdMa), including nanomaterials and multicomponent systems, present unique challenges to this framework due to their dynamic properties, life-cycle transformations, and diverse application scenarios. Steps 2 and 3 of the SSbD framework target the critical phases of production, processing, use, and disposal, requiring robust and adaptable tools to assess exposure and risks across these stages.

This study systematically reviewed 31 tools for exposure and risk assessment, categorizing them into three tiers based on complexity, data requirements, and usability. The review identified gaps in tool availability,

with some tools outdated or inaccessible, and highlighted promising advancements from recent projects, although some are not yet widely available to the public. Additionally, challenges persist in addressing AdMa-specific behaviours, including aggregation, dissolution, and transformations throughout their life-cycle.

The findings underscore three critical challenges: persistent data gaps, the need for better integration of tools across life-cycle stages, and the limited accessibility of high-complexity tools for smaller enterprises. Overcoming these obstacles will require the development of adaptable methodologies, enhanced data-sharing practices, and cross-sector collaboration to make tools more user-friendly and broadly applicable. This study provides a comprehensive assessment of tool readiness and highlights actionable insights to support the effective implementation of SSbD principles for AdMa, paving the way for safer and more sustainable innovation.

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6.05.C Implementing Holistic SSbD Approaches to Chemicals and Materials: What Do Academia, Industry, Regulators and Policymakers Propose?

6.05.C.T-01 A Pragmatic Approach to Merge Concepts of Risk Assessment and Life Cycle Assessment Toward Operationalization of SSbD: A Case Study

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Despite some guidance from JRC on SSbD being available, a final framework has not been published yet. Therefore, experience from a case study could contribute to standardisation. We propose a pragmatic methodology to find a middle ground between SSbD dimensions (safety, sustainability and performance) by integrating concepts of risk assessment, life cycle assessment and lessons learned from working with companies across different sectors. Our approach is guided by the draft JRC framework and methodological guidance, the CEFIC SSbD guidelines and the roadmaps developed by multiple stakeholder consortia under EU's framework for R&D&I. We reviewed the information available in the current SSbD frameworks, guidelines and roadmaps and compared this with in-house experience to identify indicators, criteria and tools deemed applicable at each phase of the innovation process, by increasing the Technology Readiness level (TRL) of the portfolio solution.

The SSbD methodology was applied on different paint formulations containing organic and inorganic photostabilizers meant to prevent photodegradation of wood. The aim is to select the paint with the best compromise in terms of safety, sustainability and performance for wood protection by comparing with market standards, and explaining in a transparent way how a final decision on the best paint option was derived, avoiding absolute scoring. Different scenarios are analysed by considering the following variables: the complexity of the paint in terms of composition, data availability in common databases, company in-house tools and expertise, budget constraints tied to the stage of innovation and the likelihood of the product reaching the market.

Our case study highlights the importance of SSbD criteria harmonization, SSbD tools flexibility depending on the case study in analysis, the innovation stage and company-specific in-house availability. Transparency is key in the evaluation to fully operationalize SSbD. We identified the following challenges: criteria harmonization across sectors, lack of standardised weighting approaches, difficulty to get high quality data for LCA allowing differentiation of SSbD versions at early stages, lack of automation tools available to companies in house and hard communication across the supply chain.

6.05.C.T-02 Safe-and-Sustainable-by-Design Advanced Materials: Application of the HARMLESS Approach to Imogolites for Agricultural Application

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In this practical case study, functionalised imogolites developed for agricultural purposes (imo-OH, imo-CH₃, Cu-imo-CH₃) are taken through a flexible stage-gate innovation process and used to test the

HARMLESS SSbD Decision Support System (DSS) at early innovation stages.

All lifecycle stages and two flexible innovation stages were considered in the development of the DSS. First, at ideation and business case, the AMEA tool enables the categorisation of the material under study and the verification that the DSS is applicable. Then, still at these earliest stages of development, another tool was developed to raise Warning flags, produce design Advice and identify Screening Priorities (WASP). It consists of 12 questions addressing safety and environmental sustainability. Subsequently, at lab phase, the Alternative SSbD Inspector (ASDI) helps producing a matrix, which enables the integration of data on several material alternatives, including newly developed, conventional and benchmark materials. Based on descriptors triggered by WASP, various SSbD dimensions (i.e. performance, human safety, environmental safety, environmental sustainability) are assessed quantitatively and qualitatively to enable the selection of the best material alternative to proceed to pilot phase. The quantitative safety assessment is based on New Approach Methodologies, where available. The qualitative sustainability assessment is based on Sustainable Development Goals targets defined by the United Nations, whose descriptors have been tailored to the SSbD materials scope. The scoring system takes inspiration of the Portfolio Sustainability Assessment published by the World Business Council for Sustainable Development (WBCSD).

Results show that the Cu-imo-CH₃ version raises most concern, as Cu is a toxic component and a critical raw material, and this imogolite version raises more flags regarding exposure through inhalation. Imo-OH and imo-CH₃ are further differentiated using ASDI, with imo-CH₃ showing the highest potential to be taken to pilot phase. Comparing these newly developed materials to conventional materials (i.e. Cu salts), ASDI also shows the benefits of developing functionalised imogolites on SDG 2, 12 and 14. By weighting information across several aspects of SSbD, the HARMLESS DSS results enable decision making by the material developer, influencing their design at early innovation stages.

6.05.C.T-03 Build on The International Fragrance Association (IFRA) Case-Study on methyl salicylate to Enhance the European Union Safe and Sustainable by Design (SSbD) Framework in 2025

Annika Batel, BASF, Germany

In 2022, IFRA decided to proactively test the EU SSbD framework, during the 2-year testing period (by end 24).

Goals were: Identify challenges, trade-offs & gaps in the SSbD framework, focusing on safety-sustainability integration, data availability, and shared methodological understanding within industry & supply chains; Highlight necessary improvements before 2025 s framework revision; Assess the framework's applicability to complex substances and value chains, address gaps, and consider simplifications to support holistic approach.

A case study was conducted on methylsalicylate, a common fragrance ingredient sourced both naturally and synthetically and used in different applications. The two sources of production (natural & synthetic) and two end products (a cosmetic & a detergent) were analyzed and compared. The study evaluated the applicability of the draft SSbD framework across its first four steps. Social and economic sustainability aspects were outside the study's scope.

Step 1: Using ECHA database information, methyl salicylate was identified as a substance of concern. The hazard assessment was straightforward due to its REACH registration and harmonized classification, but such assessments could be more complex for unregistered or lower-tonnage substances. Step 2: Both synthetic and natural methyl salicylate manufacturers were contacted to evaluate worker exposure risks related to chemical handling and intermediate substances. In both production processes, potential risks could be identified where mitigation could improve safety. Step 3: Safety for human and environmental health was assessed based on end use in consumer products. Both uses were identified as safe for human health and the environment. Step 4: A life cycle assessment was completed for both sources and both applications, highlighting the differences of potential impact categories depending on the sourcing of the substance.

General key findings include significant data challenges (e.g., gaps, low-quality, confidentiality), limited supply chain expertise for safety and sustainability, and methodological challenges. There was also uncertainty in selecting appropriate sustainability assessment references, increased workload complexity due to typical fragrance compositions, and a need for clearer guidance within the SSbD framework. Provide recommendations to improve the EU SSbD framework in 2025, aiming to make it a practical reference for safe and sustainable innovation.

6.05.C.T-04 Application of the Safe and Sustainable by Design (SSbD) Principle in the Development of Circular and Recyclable Composites for Automotive and Aerospace Sectors

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Innovation in materials and processes is leading to high performance multicomponent and multifunctional advanced materials. The integration of the Safe and Sustainable by Design (SSbD) framework in the early stages of this innovation process will speed their development, ensuring that risks and environmental impacts are not a drawback.

graphene-based materials (GBMs), and particularly graphene and graphene oxide (GO) have gained interest to enhance multi-functionality of structures and components used in high demanding applications, as in the aerospace and automotive industries, and often enable combination of materials with digital technologies. Examples of the benefits linked to the application of advanced additives in these sectors include: the reduction of the vehicle weight (e.g. embedding circuits to replace wires); the improvement of the efficiency of the production process (e.g. substituting critical raw materials and halogenated additives, and reducing energy demand in processing composites by enabling the use of infrared curing); the optimization of the use conditions (e.g., integrated structural health monitoring sensors into structural matrix and enhancing the flame retardancy of the matrixes).

The use of additives does not only have a significant impact on the material functionalities and performances, but also on its safety and sustainability along the whole life cycle.

In this context, establishing and implementing a SSbD strategy is key to understand, at early stages of the material development, the balance between the benefits foreseen and potential risks that the adoption of GBMs additives could bring.

The REPOXYBLE EU funded project (2023-2026, GA 101091891) utilizes GBMs to enhance the thermal and electrical conductivity of epoxy-based polymer composites, reduce energy demands in composite processing (e.g. infrared curing to harden the resins), for the automotive and aerospace industry. Similarly, The SUNSHINE project investigates GO production and functionalization to improve flame retardancy in thermoplastic composites for printed electronics and other sectors. The projects involve some of the largest developers and producers of GO in Europe.

The presentation will explain SSbD practices that are being developed and implemented in these projects for the processing, production and integration of GBMs in advanced composites.

6.05.P-Tu450 Methodological Approaches and Applications for Early TRL SSbD: Case Study on Upconverting Particles Used in Biocidal Coatings

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The Safe and Sustainable by Design framework can be applied at any stage; from the innovation and design phase to the fully developed phase. Its iterative approach encourages the assessment to be improved as more data, and certainty become available during the innovation process. For a fully developed SSbD, high level of data is required. Although SSbD is possible for early Technology Readiness Levels (TRLs), more solid approaches are needed to address the data limitations at these stages. On the other hand, biocides, widely used in healthcare, food safety, and agriculture present challenges in applying SSbD due to their functionality that also provide benefits. An example of an early TRL biocide is upconverting particles, which convert lower-energy light into higher-energy UV radiation through a process called upconversion. Rare earth element containing materials (or hosts) efficiently perform this conversion. Recently, Pr³⁺ doped materials were reported to efficiently emit in the UV range which makes them useful to be used as biocides. In general, the SSbD framework with some specific considerations was followed throughout the study, applying a recently developed biocide-specific SSbD framework. First, the specific data requirements and gaps were identified for each step. Secondly, various methodological approaches were evaluated and then adjusted where needed in order to address these needs, focusing on ways to minimize gaps and effectively generate the required data that fits the specific chemical. Lastly, the case study of upconverting particles was applied to demonstrate the feasibility of conducting SSbD at early TRLs. Our results highlight the need for refining SSbD methodologies for early TRL applications, ensuring that early-stage chemicals can be assessed for safety and sustainability. This work provides a foundation for integrating early TRL chemicals into sustainable innovation processes.

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6.05.P-Tu451 Safe and Sustainable by Design Strategies: A Hotspot-based Approach for Advanced Materials

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The Safe and Sustainable by Design (SSbD) framework for chemicals and materials was established to guide the transition envisioned by the European Green Deal Chemical Strategy for Sustainability to a toxic-free environment. It was developed within the EC Joint Research Centres and further described in the European Commission's (EC) Recommendation for establishing a European assessment framework for SSbD. To operationalize this framework, the H2020 SUNSHINE project has developed a tiered SSbD assessment methodology, which was tested in a suite of advanced multi-component nanomaterials. This approach enables the assessment of safety and sustainability aspects at each stage of product development from a lifecycle perspective. Tier 1 consists of a qualitative self-assessment questionnaire designed to evaluate the early stages of R&D and product optimization within the innovation process. The results highlight critical "hotspots" concerning safety and sustainability across the entire lifecycle of the target materials. In Tier 2, a more comprehensive and detailed analysis is conducted to address and further investigate these identified concerns prior to their release on the market. This is done through Chemical Safety Assessment, Lifecycle Assessment, Lifecycle Costing, and Social Lifecycle Assessment methodologies. The result is a holistic Life Cycle Sustainability Assessment of the materials / products investigated. Tier 2 played a crucial role in validating the results from Tier 1 and in addressing the identified "hotspots." This tiered approach was applied to four SUNSHINE industrial case studies through an iterative process. The findings demonstrate that Tier 1 was highly effective in supporting the early stages of material development by identifying potential safety and sustainability challenges during the R&D phase, when adjustments to the production process were still possible and cost-effective, and there was flexibility in choosing alternative design options. This has highlighted the added value of the SUNSHINE SSbD approach in enabling companies to assess the safety and sustainability performance of their products in a straightforward, targeted, and cost-effective way. This approach can significantly enhance the competitiveness of these industries in the market, while also driving the development of more environmentally friendly nanotechnologies that offer substantial societal and economic benefits.

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6.05.P-Tu453 Engaging Stakeholders and Applying Safe and Sustainable by Design (SSbD) Principles in the PROTEUS EU Project

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The initial focus of SSbD has been on chemicals, this study will apply the application of SSbD principles and LCSA to evaluate a novel production method in the context of kelp harvesting and biorefining, emphasizing cross-disciplinary collaboration, transparent communication along the value chain, and an iterative scientific process throughout the EU funded project: PROTEUS. The integration of these dimensions within the SSbD framework will be supported by stakeholder involvement and data transfer between industry and research partners to foster shared understanding and alignment on sustainability and safety priorities.

The framework presented integrates SSbD and LCSA. As the project progresses, experience and interactions between stakeholders will result in the development of this framework through an iterative process. The application of this SSbD and LCSA framework to a value chain where the innovation is the reduction (or removal) of hazardous chemicals means that it is not immediately obvious what benefits are derived from using an SSbD framework, beyond documenting the improvements possible with the innovation being developed. The authors maintain that there are benefits in integrating these approaches that can also be applied to systems where a value chain innovation is to be realised that reduces, or eliminates chemical hazard. The benefits of this approach will be explored and documented during the PROTEUS project. Stakeholder engagement will initially involve the value chain actors that are partners in the EU Horizon project. However, as the work progresses further stakeholders will be involved. The work presented will show the stakeholder involvement steps planned during the Proteus project timeline.

This work aims to highlight the potential of LCSA as a cornerstone for implementing SSbD principles in innovative production processes. By facilitating interdisciplinary collaboration and aligning diverse stakeholder interests, LCSA supports the paradigm shift towards safe and sustainable innovation. Future

efforts in the Proteus project will focus on refining the LCSA/SSbD framework, engaging stakeholders and continuous iterative improvement.

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6.05.P Implementing Holistic SSbD Approaches to Chemicals and Materials: What Do Academia, Industry, Regulators and Policymakers Propose?

6.05.P-Tu438 Exposure Assessment Along the Life Cycle of Nanocomposites

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Carbon nanotubes in general have outstanding properties that enable new applications such as high-performance concrete, super capacitors, sensors, improved conductive electrodes and inks, polymer nanocomposites, etc.. In polymers, the carbon nanotubes are used as additives to reinforce and enhance mechanical properties or to enable electrical and thermal conductivity. However, rigid, stiff and thin multi-wall carbon nanotubes can present asbestos-like hazards if they are released and inhaled, for example through abrasion from products. To minimize health risks as far as possible, the aim is to optimise their design according to the principle of Safe and Sustainable by Design, with a short length and low stiffness to enable them to be transported by macrophages in the lungs. We propose that this can be achieved with single-wall carbon nanotubes. Yet, this hypothesis is to be confirmed in release and toxicity studies, for which release, and more toxicity studies are still needed. In this context, we conducted two case studies to investigate possible possible released of single-wall carbon nanotubes from polymer nanocomposites. Potential exposure to airborne single-wall carbon nanotubes was studied during two laboratory-scale case studies of epoxy resins and lithium-ion batteries containing a Single wall carbon nanotube type in the polymer matrix and conductive electrode material, respectively. The single-wall carbon nanotubes, marketed under the brand name TUBALL, exhibits a distinctive morphology and behaviour during the life cycle stages in comparison with other carbon nanotube types. This renders some standardised test models inapplicable. The data generated from the case studies conducted under the European Commission projects DIAGONAL and SUNSHINE for the purposes of exposure assessment and Safe and Sustainable by Design indicate that the nanomaterial TUBALL is safe for use. To further confirm these findings, it is important to assess all the life cycle stages of single-wall carbon nanotubes and their environmental pathways and potential exposure routes. Regarding Safe and Sustainable by Design, the objective is to ensure responsible and safe use with meaningful risk management.

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6.05.P-Tu439 Implementation of Safe and Sustainable by Design (SSbD) Approach in the Development of Bio-based Products

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Safe and Sustainable by Design (SSbD) is a central component in the European Chemicals Strategy for Sustainability and has the potential to be the tool to support industry through innovation of new products in the transition towards climate neutrality and a less toxic environment. However, a common understanding of what SSbD is in concept detail and in practice is still under discussion and development. In this study, we examine the applicability and effort for implementation of the SSbD framework to several bio-based products and materials across several industrial sectors. In our assessment, we include Hazard Assessment looking at the intrinsic properties of the compound or material in order to understand its hazard profile (human health, environment and physical hazards). Human Health and Safety Assessment looking at the adherence of the process to EU directives or regulations, Environmental and Human Health Risk at the final application or material looking at whether the use of a compound or material in its final application poses any risk to human health or the environment. Further, Environmental Impacts Evaluation along the entire life cycle, covering the assessment of the environmental sustainability aspects of the compound or material in question, focusing on its product environmental footprint. Finally, and complementing all the above activities, a semi-quantitative social life cycle assessment (sLCA),

based on the UNEP SETAC recommendations and the recently published ISO standard, in the key impact categories: employment, occupational health and safety, community well-being, and societal benefits will be conducted. Where possible, existing components of the Partnership for the Assessment of Risks from Chemicals (PARC) SSbD toolbox were taken into account in our study and further tool elements will be incorporated into our assessment upon integration into the toolbox. In order to determine the potential environmental benefits of bio-based products and materials, a preliminary assessment has been performed, based on an extensive review of LCA studies complemented by information from commercial LCA databases. In this analysis, the favorable environmental performance of bio-based products has been proven by comparing it with conventional production.

6.05.P-Tu440 A Safe & Sustainable by Design R&D Pipeline Using In Silico Methodology

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As we move towards the framework of Safe & Sustainable by Design (SSbD) products, companies increasingly incorporate environmental and human health protection considerations into their Research & Development (R&D) pipeline. As a result, New Approach Methodologies (NAM) are required to process, generate or predict (eco)toxicological and environmental effects of newly discovered families of compounds. Over the years, in silico predictions have garnered more recognition thanks to a greater understanding of the available methods as well as significant improvements in the depth and versatility of the tools now on the market. Use of in silico methods, (quantitative) structure activity relationship (Q)SAR model, to predict the (eco)toxicity and/or environmental fate of a substance is the logical choice to identify new compounds due to the speed and relative low cost when bulk predictions of numerous substances are required. A project requested by Chevron Phillips Chemical was undertaken by KREATiS to assess the toxicological effects of a wide range of chemicals of interest. Based on both technical and economical considerations, it was decided to focus on the most critical effects for human health: carcinogenic, mutagenic and reprotoxic (CMR) profile and skin sensitisation. The first phase of the project was to group substances into chemical families based on structural and mechanistic insight. The second phase was the prediction of CMR and skin sensitisation for selected substances from each group, using available (Q)SAR tools and an in-depth review of these candidates. In the third phase, newly designed substances as well as high potential candidates previously grouped in high interest categories were reviewed. The latter having been characterized as having properties of relatively low concern for human health. Thus, using a battery of predictive in silico tools to estimate the relative toxicity of a large batch of promising compounds in the early-stage of R&D can help screen for the less desirable compounds and identify the most promising substances. A full scheme on how this was achieved is described in this poster.

6.05.P-Tu441 Redesigning for the Future: Applying Safe and Sustainable by Design (SSbD) Principles in High-Impact Chemical Industries

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Adopting Safe and Sustainable by Design (SSbD) principles presents challenges for companies, requiring a shift from market-ready products back to pre-market R&D stages. This challenge is intensified by a complex and evolving regulatory landscape, especially in sectors like food contact materials, aviation, and pharmaceuticals. Without clear incentives, companies often hesitate due to high R&D costs, potential new regulatory approvals, and the risk that minor product changes might lead to customer rejection and financial losses.

The SSbD draft framework by the Joint Research Centre (JRC) provides a structured yet inherently stepwise approach. This sequencing can hinder efficient data integration, as data from each step must be combined across stages, often creating lengthy assessments. Additionally, data are often unavailable, immature, non-comparable, outdated, or incomplete. SSbD assessments span numerous disciplines, and no single expert covers all the knowledge required. A critical question is whether SSbD can be made more accessible for industry. Specifically, could product developers, even without deep SSbD expertise, use simplified tools to identify essential tasks and expert inputs, lowering the activation barrier for SSbD implementation without unrealistic black box expectations?

Our work demonstrates the SSbD framework's applicability by developing alternatives for three key chemical classes plasticizers, flame retardants, and surfactants progressing from Technology Readiness Level (TRL) 6 to incorporation in consumer goods at TRL 7. Central to our approach is exploring interconnections between SSbD's five steps, from initial safety assessments to broader sustainability

evaluations, and visualizing these links to support effective tool selection across SSbD stages. An overarching question remains: would an SSbD certification a "seal" of safety and sustainability justify a higher product price for consumers? For companies, SSbD investment requires more than regulatory compliance it demands alignment of sustainability efforts with market acceptance. How much standardization of SSbD is needed to create an incentive for the value chain? By examining whether such efforts could bridge the gap between innovation and economic viability, we aim to address the initial hesitations companies face. Early findings from this ongoing work will be presented, highlighting the holistic application of SSbD in redesigning established products.

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6.05.P-Tu442 Screening Sustainability Assessment of Innovative Bio-Based Solution for Art Restoration

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Within the HEU GREENART project, an assessment approach was developed to evaluate the safety and sustainability of innovative solutions for art restoration, including protective coatings, foams and packaging materials, consolidants, gels and cleaning fluids, and green tech sensors. This approach, developed taking in consideration the Safe and Sustainable by Design (SSbD) Framework and the Nanorestart Framework, is structured into three iterative stages. Stage 1 involves the Hazard Assessment of innovative ingredients and formulations. Stage 2 includes a Screening Sustainability Assessment. Stage 3 focuses on assessing the sustainability performance of the most promising solutions identified in the earlier stages, through Life Cycle Assessment (LCA). This study focuses on the stage 2 of the GREENART approach: the Screening Sustainability Assessment, developed during the second year of the project and already used to guide the decision-making process. This assessment is conducted through a questionnaire structured around three main aspects: Functionality, Safety and Sustainability. The three aspects are assessed across the entire life cycle of GREENART's innovative products and benchmarks. Each aspect is assessed through specific criteria, providing measurable indicators of sustainability performance. The criteria help in determining the significance and impact of each aspect within the life cycle stages. Finally, the assessment incorporates targeted questions designed to evaluate compliance with the established criteria. Each question has five possible answers, representing the level of satisfaction with a specific product solution, ranging from "excellent" to "bad". These scores are then averaged to visualize the overall results across the different life cycle stages and sustainability aspects.

The safety aspect is assessed by combining the Hazard Classification from Stage 1 of the GREENART approach with an Exposure Classification, inspired by the ProScale method, within the GREENART Control Banding Matrix.

The results of the Screening Sustainability Assessment will be presented for one case study, selected from GREENART's innovative products and related benchmarks.

This approach aligns with the SSbD framework, ensuring compliance with evolving European and international chemical safety regulations while continuously improving the safety and sustainability profile of these products.

6.05.P-Tu443 From Ambition to Action: Navigating Obstacles and Opportunities of 'Safe and Sustainable by Design'

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With the introduction of Safe and Sustainable by Design (SSbD) concept, momentum is being created in the European Union (EU) to shift from reactive (mis-)management of chemicals and materials to a more proactive design and assessment approach to prevent pollution issues. The concept holds the potential to steer the innovation process toward a green and sustainable industrial transition, promote the reduction and substitution of substances of concern, and minimize health and environmental impact throughout the life cycle of chemicals and materials. To operationalize the SSbD concept, the European Commission has recommended a framework, but many questions remain open regarding its feasibility and implementation. The aim of this work was to deepen the current discussion on SSbD towards an effective operationalization. We critically analyse and discuss how the by design aspect and the safety and sustainability assessments of SSbD constitute a unique opportunity for the green transition. We evaluate

obstacles and highlight opportunities of operationalizing the EU SSbD framework in a refined manner. Our analysis indicates that the EU SSbD framework, despite its potential, is unlikely to fully achieve its ambitions in its current form. In particular, suitable assessment methods are not yet available for many aspects, and the complexity and data requirements of the SSbD framework may hinder widespread adoption or result in paralysis by analysis. Moving forward, a more realistic, agile framework, accompanied by clear, simplified methods and robust support for stakeholders, should be developed to ensure the SSbD principles are fully integrated into practice, leading to truly safer and more sustainable chemicals and materials. We further highlight opportunities to address identified gaps, establish an improved framework, and enhance its operationalization.

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6.05.P-Tu444 Chemical Assessment Frameworks and their Alignment to the EC Safe and Sustainable by Design Framework: An Initial Exploration

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The HEU GREENART project proposes innovative chemicals and materials for the conservation and restoration of Cultural Heritage. All GREENART solutions are assessed in terms of safety and sustainability performance. The assessment begins with the use of green metrics by product developers. In particular, the Global Score method from CSGI is a tool to evaluate chemicals, specifically solvents and surfactants. It incorporates available green metric scores, derived from several chemical frameworks (i.e., CHEM 21, Sanofi, GSK, Pfizer, Astrazeneca). Following this, a more thorough evaluation is underway in the project to assess the safety and sustainability of the GREENART solutions throughout their entire lifecycle. This novel approach comprises a Hazard Assessment, a Screening Sustainability Assessment and a full Life Cycle Assessment (LCA). The JRC's Safe and Sustainable by Design (SSbD) Framework and the Nanorestart Framework are at the foundation of this approach. The goal of this study was to explore the chemical frameworks considered in the Global Score method and to evaluate their alignment with the SSbD Framework in terms of procedure and design principles. The evaluation proceeded gradually, starting from the SSbD principles proposed to support product developers during the design phase. These include aspects related to safety and resource use, considering the whole life cycle, and related indicators. Some of these indicators are also evaluated in the chemical frameworks included in the Global Score method. Next, the alignment of the green chemistry indicators from chemical assessment frameworks with the five steps of the SSbD framework is evaluated. The highest alignment has been observed in Step 1 for Hazard Assessment and Step 2 and 3 for Safety Assessment. Indeed, most green chemistry indicators consider intrinsic hazardous properties of chemicals and potential risks for workers during production and final application phases. Step 4 for Environmental Sustainability Assessment is only partially covered by the chemical frameworks under study. Although some specific indicators exist for LCA, it is unclear how the LCA behind them is performed, and which data are considered. Step 5 for Social and economic sustainability assessment is the least addressed. This work is expected to support the application of principles that extend beyond traditional green chemistry, aiming to enable science-based decision making within the SSbD era.

6.05.P-Tu445 Application of SSbD Concepts to the Development of Bio-based Epoxy Compounds for Polymer Coatings

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Two-component epoxy represents a developed group of chemicals having a broad use in building and coating applications worldwide. Although they contribute to sustainability by protecting and expanding lifetime of products and installations, they are almost exclusively derived from fossil resources and based on compounds like bisphenol A (BPA) that present a threat to health and the environment. The NordiCoats project aims to lay the basis for the development of new epoxy-based solutions that are bio-based, safer in use, potentially recyclable, and with performance levels close to relevant benchmarks.

This is achieved by assessing the sustainability and safety of the new bio-based compounds and formulations. Here, we present a comparison of the aqueous leaching of 20 different candidate coating formulations and link this to the bulk material composition to identify those chemicals which are preferentially released. Non-target and suspect screening were performed using both thermal desorption-pyrolysis GC-MS and GC×GC-MS on the bulk coating material and aqueous leachate solvent extracts. By using a combination of known ingredients and mass spectral library matching to provide lists of the main components in the bulk coating materials and leachates, existing environmental toxicity and human toxicity data were retrieved from databases and used to perform a risk assessment. This approach allowed us to identify those coating components that exhibited the highest leaching potential and toxicity, thereby indicating which coating formulations had the least impact on humans and the environment.

6.05.P-Tu446 A Multi-actor Approach to Facilitate the SSbD Implementation in SURFs UP Bio-based Value Chains

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Currently only 4% of the surfactant market share consists of compounds that are 100% based on biomass (fully biobased surfactants), while there is an urgent need for non-hazardous Safe and Sustainable by Design (SSbD) biosurfactants (BSs). The biosurfactant market is expected to grow at a CAGR (Compound Annual Growth Rate) of between 5.2 and 10.5% between 2022 and 2028. However, the diversity of the currently available safe and sustainable biosurfactants is not at all sufficient to meet the market needs: diversity is key. SURFs UP proposes a (integrated) combination of biological & green chemical production routes from (waste) biomass second generation (2G) feedstocks and following SSbD criteria which will allow a dramatic transformation of the surfactant market by offering the broad diversity of safe and sustainable alternatives at a reasonable cost that the market is looking for. SURFs UP will bridge this gap by demonstrating optimized and cost-efficient processes for 9 SSbD biosurfactant products that will be formulated in prototypes in home- and personal care and agrochemical applications. These ingredients will be fully characterized and evaluated according to the EC SSbD Framework criteria. Finally, the production of SSbD BSs will be scaled up and used to demonstrate prototype formulations in home care, personal care, and biopesticide applications. SURFs UP project activities implement a multi-actor approach according to the quadruple helix innovation model, where science, policy, industry, and society are involved in the technology development and innovation process. This ensures that all the relevant actors and end-users in the bio-based value chain are appropriately involved. In SURFs UP a decision-making process will be implemented (e.g., stage to gate) along the supply chain to promote the integration of the SSbD principles in all stages of product development, use and end-of-life. We ensure an active and iterative flow of information from the start of the project, with all the actors involved in the products development, through meetings and workshops. Important information will be produced throughout the project which will be analyzed in an integrated way.

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6.05.P-Tu447 The SUNRISE SSbD Integrated Impact Assessment Framework

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As part of the European Green Deal, the Chemicals Strategy for Sustainability and the Zero Pollution Action Plan highlight the critical need for a Safe and Sustainable by Design (SSbD) approach to chemicals and materials. To drive this transformation, the European Commission (EC) has recommended the establishment of a European assessment framework for SSbD of chemicals and materials, based on a holistic approach developed by the EC's Joint Research Centre. To facilitate the practical adoption of the EC-JRC framework by stakeholders, particularly within the industry, the Horizon Europe SUNRISE project has been funded to develop integrated methodologies for assessing the health, environmental,

social, and economic impacts of products enabled by advanced materials (AdMa). The SUNRISE project combines the bottom-up development of methods and tools for assessment of health, environmental, social and economic impacts with their top-down integration in an overarching Integrated Impact Assessment Framework (IIAF). This framework is designed to support SSbD decision-making along supply chains and lifecycle stages of AdMa and their products. The process of developing and testing the IIAF in cocreation with the stakeholders within a trusted real-world environment will improve our understanding of stakeholders needs, perceptions and relevant SSbD aspects to be considered, along with potential safety and sustainability trade-offs. This new knowledge will be transferred to regulators and policy makers at EU and national level to support them in the implementation of SSbD-related policies for chemicals and materials. The IIAF is composed of 4 assessment Tiers, fully aligned to the 5 steps of the EC-JRC SSbD framework, where each tier corresponding to an integrated methodology (supported by a toolbox) for health, environmental, social and economic impact assessment targeting different groups of users at different stages of the innovation process and requiring a different level of data and expertise. The implementation of the IIAF will ensure better regulatory compliance for AdMa based products and can have an impact on shortening the time of new materials/products to reach the market, thereby supporting the Green Deal policy objectives for the transformation of the EU into a modern, resource-efficient and competitive economy.

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6.05.P-Tu448 SEARCULAR: Towards the Practical Implementation of a Circular Economy for Fisheries

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Every year, more than eight million metric tonnes of plastic are discharged into the oceans. Among these, 600,000 to 800,000 metric tonnes of Abandoned, Lost, or Discarded Fishing Gear (ALDFG) contribute significantly, representing about 10% of marine litter by weight, according to the UN Food and Agriculture Organization. Studies indicate that ALDFG makes up to 70% of all oceanic macroplastics mass, and eventually break down into micro- and nanoplastics, posing serious risks to marine ecosystems. The SEARCULAR project addresses these environmental challenges by creating Safe and Sustainable by Design (SSbD) circular solutions for fishing gear, aimed at reducing both marine litter and the formation of micro- and nanoplastics. SEARCULAR's work includes developing four sustainable, near-market, circular solutions to reduce the ecological impact of fishing gear. Three of these initiatives focus on replacing conventional plastics with SSbD alternatives:

1. Creation of dolly ropes made from recycled polyamide (rPA) derived from discarded fishing nets.
2. Development of seine ropes from certified marine-biodegradable materials.
3. Eco-design of drifting Fish Aggregating Devices (dFADs) made of organic materials that biodegrade at marine environments.

The fourth strategy addresses end-of-life (EoL) fishing gear management by promoting a replicable system at harbors for processing this gear into reusable plastic through pyrolysis, thus converting discarded plastic into a resource.

These innovative approaches aim to support the fishing industry and related sectors in reducing their environmental footprint by embracing circular strategies, utilizing numerical modeling, and establishing replicable EoL management practices. The SEARCULAR project will rigorously assess and validate the sustainability of each alternative, engaging stakeholders from industry, science, policy, and non-governmental organizations (NGOs) to facilitate acceptance and behavioral change within the fishing sector. Additionally, SEARCULAR will inform policymakers of critical challenges and best practices to help enable the broad adoption of sustainable practices across Europe. SEARCULAR has received

funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 101112852.

6.05.P-Tu449 Key Performance Indicators in SSbD: A Transparent and Data-Driven Methodology

Irini Furxhi and Anna Costa, CNR ISSMC, Italy

"The Safe and Sustainable by Design (SSbD) framework by the Joint Research Centre (JRC), aims to establish criteria and evaluation procedures for chemicals, (nano)materials, products, and processes. This framework emphasizes the assessment of the entire life cycle of compounds, incorporating human and environmental safety aspects alongside environmental and economic sustainability dimensions. The overarching goal is to stimulate sustainable research and innovation that transcends current regulatory requirements.

ASINA project employed the SSbD framework in nanotechnology case studies to identify material design alternatives early in the innovation process. This approach reduced the potential for hazardous material release and minimized hazard levels, while ensuring functional performance for intended applications. This presentation will take the concept further by introducing a data-driven methodology for merging diverse datasets to derive a unified indicator. Specifically, we will:

1. Present measurable, quantitative, and qualitative indicators, defined for various aspects (e.g., health and environmental impact) and measured through experimental or modeling methods.
2. Highlight the role of Key Decision Factors (KDFs), which are modifiable by the designer to influence the final indicators, enabling flexibility in the "re-design" process.
3. Explore Key Performance Factors (KPFs), which influence outcomes but are not under the designer's control.

By providing indicators and linking them to their respective KDFs and KPFs, this methodology offers a robust starting point for future SSbD implementations and data management strategies. The approach ensures transparency and fosters alignment with ongoing and future projects striving toward similar objectives, inspiring sound scientific practices. Ultimately, by developing integrated indicators, we aim to streamline the SSbD evaluation process, supporting a harmonized, data-driven approach to sustainable innovation across material and process life cycles.

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6.05.P-Tu450 Methodological Approaches and Applications for Early TRL SSbD: Case Study on Upconverting Particles Used in Biocidal Coatings

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The Safe and Sustainable by Design framework can be applied at any stage; from the innovation and design phase to the fully developed phase. Its iterative approach encourages the assessment to be improved as more data, and certainty become available during the innovation process. For a fully developed SSbD, high level of data is required. Although SSbD is possible for early Technology Readiness Levels (TRLs), more solid approaches are needed to address the data limitations at these stages. On the other hand, biocides, widely used in healthcare, food safety, and agriculture present challenges in applying SSbD due to their functionality that also provide benefits. An example of an early TRL biocide is upconverting particles, which convert lower-energy light into higher-energy UV radiation through a process called upconversion. Rare earth element containing materials (or hosts) efficiently perform this conversion. Recently, Pr³⁺ doped materials were reported to efficiently emit in the UV range which makes them useful to be used as biocides. In general, the SSbD framework with some specific considerations was followed throughout the study, applying a recently developed biocide-specific SSbD framework. First, the specific data requirements and gaps were identified for each step. Secondly, various methodological approaches were evaluated and then adjusted where needed in order to address these needs, focusing on ways to minimize gaps and effectively generate the required data that fits the specific chemical. Lastly, the case study of upconverting particles was applied to demonstrate the feasibility of conducting SSbD at early TRLs. Our results highlight the need for refining SSbD methodologies for early TRL applications, ensuring that early-stage chemicals can be assessed for safety and sustainability. This work provides a foundation for integrating early TRL chemicals into sustainable innovation processes.

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6.05.P-Tu451 Safe and Sustainable by Design Strategies: A Hotspot-based Approach for Advanced Materials

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The Safe and Sustainable by Design (SSbD) framework for chemicals and materials was established to guide the transition envisioned by the European Green Deal Chemical Strategy for Sustainability to a toxic-free environment. It was developed within the EC Joint Research Centres and further described in the European Commission's (EC) Recommendation for establishing a European assessment framework for SSbD. To operationalize this framework, the H2020 SUNSHINE project has developed a tiered SSbD assessment methodology, which was tested in a suite of advanced multi-component nanomaterials. This approach enables the assessment of safety and sustainability aspects at each stage of product development from a lifecycle perspective. Tier 1 consists of a qualitative self-assessment questionnaire designed to evaluate the early stages of R&D and product optimization within the innovation process. The results highlight critical "hotspots" concerning safety and sustainability across the entire lifecycle of the target materials. In Tier 2, a more comprehensive and detailed analysis is conducted to address and further investigate these identified concerns prior to their release on the market. This is done through Chemical Safety Assessment, Lifecycle Assessment, Lifecycle Costing, and Social Lifecycle Assessment methodologies. The result is a holistic Life Cycle Sustainability Assessment of the materials / products investigated. Tier 2 played a crucial role in validating the results from Tier 1 and in addressing the identified "hotspots." This tiered approach was applied to four SUNSHINE industrial case studies through an iterative process. The findings demonstrate that Tier 1 was highly effective in supporting the early stages of material development by identifying potential safety and sustainability challenges during the R&D phase, when adjustments to the production process were still possible and cost-effective, and there was flexibility in choosing alternative design options. This has highlighted the added value of the SUNSHINE SSbD approach in enabling companies to assess the safety and sustainability performance of their products in a straightforward, targeted, and cost-effective way. This approach can significantly enhance the competitiveness of these industries in the market, while also driving the development of more environmentally friendly nanotechnologies that offer substantial societal and economic benefits.

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6.05.P-Tu452 Unlocking the Future: SSbD as the Sustainable Solution for the Life Science Industry **Athanasios Gkrillas¹, Karoline Wowra², Winfried Bulach², Alexandra Gastens², Lidia Ceriani³, Erwan Saouter⁴ and Florian Gruener², (1)Merck Life Science BV, Belgium, (2)Merck Life Science KGaA, Germany, (3)Merck Life Science Srl, Italy, (4)Net-Zero Impact (SAS), France**

The European Union's Chemicals Strategy for Sustainability (CSS) aims to foster a toxic-free environment by promoting the "Safe and Sustainable by Design" (SSbD) framework for pre-market chemical assessments. This framework systematically evaluates the safety, environmental impact, and socio-economic sustainability of chemicals, encouraging redesign when performance does not meet SSbD criteria. Merck's Life Science business, with over 300,000 products globally, provides essential tools, high-quality chemicals, and consumables for research, biotechnology, and pharmaceutical development. However, the complexity of Merck's portfolio presents challenges for the immediate and comprehensive implementation of SSbD at scale, as it requires extensive data and expert evaluation.

To address this, we propose a flexible application of the SSbD framework as a solution-oriented approach for assessing the safety and environmental sustainability of both new and existing Life Science products, scaling the methodology based on data availability and product prioritization. To evaluate our effectiveness regarding data, tools, and methods, we conducted several SSbD case studies across various product types, including challenging categories like polymers and articles. We faced challenges such as limited acceptance of New Approach Methodologies (NAMs) to address data gaps, variability in safety scores due to different exposure tools, difficult acquisition of tonnage information, and data availability issues from suppliers affecting Life Cycle Assessment (LCA). We identified strategies and tools to overcome some challenges, while others require further dialogue with relevant stakeholders at the European level to enhance the SSbD framework. The high data demands of the SSbD framework necessitate a pragmatic, stepwise approach, particularly for low-volume and low-hazard chemicals. We

also advocate for a unified methodology and scoring system to facilitate meaningful comparisons across companies, improving the operationalization of the SSbD framework. This work highlights the potential of the SSbD framework to enhance chemical safety and environmental sustainability in the Life Science sector while addressing the complexities of data requirements and model integration. Future enhancements and industrial-scale applications of the SSbD framework will be discussed, emphasizing the need for collaboration within the chemical industry to achieve sustainable solutions.

6.05.P-Tu453 Engaging Stakeholders and Applying Safe and Sustainable by Design (SSbD)

Principles in the PROTEUS EU Project

Cecilia Askham, Valentina Helen Pauna, Rannva Danielsen and Anna Woodhouse, NORUS, Norway

The initial focus of SSbD has been on chemicals, this study will apply the application of SSbD principles and LCSA to evaluate a novel production method in the context of kelp harvesting and biorefining, emphasizing cross-disciplinary collaboration, transparent communication along the value chain, and an iterative scientific process throughout the EU funded project: PROTEUS. The integration of these dimensions within the SSbD framework will be supported by stakeholder involvement and data transfer between industry and research partners to foster shared understanding and alignment on sustainability and safety priorities.

The framework presented integrates SSbD and LCSA. As the project progresses, experience and interactions between stakeholders will result in the development of this framework through an iterative process. The application of this SSbD and LCSA framework to a value chain where the innovation is the reduction (or removal) of hazardous chemicals means that it is not immediately obvious what benefits are derived from using an SSbD framework, beyond documenting the improvements possible with the innovation being developed. The authors maintain that there are benefits in integrating these approaches that can also be applied to systems where a value chain innovation is to be realised that reduces, or eliminates chemical hazard. The benefits of this approach will be explored and documented during the PROTEUS project. Stakeholder engagement will initially involve the value chain actors that are partners in the EU Horizon project. However, as the work progresses further stakeholders will be involved. The work presented will show the stakeholder involvement steps planned during the Proteus project timeline.

This work aims to highlight the potential of LCSA as a cornerstone for implementing SSbD principles in innovative production processes. By facilitating interdisciplinary collaboration and aligning diverse stakeholder interests, LCSA supports the paradigm shift towards safe and sustainable innovation. Future efforts in the Proteus project will focus on refining the LCSA/SSbD framework, engaging stakeholders and continuous iterative improvement.

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6.05.P-Tu454 Early-stage Application of the SSbD Framework on PFAS Alternatives for Textile and Packaging Sectors

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PFASes accumulate in ecosystems and cause severe health and environmental issues. In response, the European Union will be restricting PFASes by means of a REACH restriction (Restriction proposal currently under evaluation by the European Chemicals Agency (ECHA) committees). Balancing performance requirements with health and environmental considerations when developing PFAS-free coatings is a complex process that requires innovative approaches in both material science and product design. The main objective of the ZeroF project is to develop and validate two novel PFAS-free hybrid coating formulations; carbohydrate fatty acid esters (CFAEs) for both packaging and textiles and silicate-based organic-inorganic hybrid materials (ORMOCER®) for upholstery textiles. To obtain the desired omniphobicity, different strategies based on chemical modifications, the addition of performance additives and structuration effects have been studied. The project is using the Safe and Sustainable by Design (SSbD) framework published for chemicals and advanced materials by the Joint Research Centre to guide the design of these coatings along their development stages. In the early phase of innovation, safety,

sustainability and cost screenings of materials and chemicals envisaged for formulations (e.g. precursors, solvents, and additives) were conducted. Data gaps and limited applicability of in silico tools were challenging for the assessment of the PFAS-alternatives themselves. Complex modeling approaches (e.g. using mixture and transformation considerations) are deployed to overpass these challenges and will be complemented by toxicity data generated for the most promising PFAS-alternatives using new approach methodologies at a later stage of the innovation. Together, these data should allow the risks for workers, consumers and the environment associated to PFAS-alternatives synthesis, processing, use and end of life to be compared to those estimated for PFAS. ZeroF combines SSbD, technical developments and modelling in iterative evaluations to create upholstery textile and food packaging prototypes that are safer and more sustainable while meeting the required oil and water barrier repellencies.

6.05.P-Tu455 How Can Innovation Management Support SSbD?

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The Safe and Sustainable by Design (SSbD) framework is a transformative approach to innovation, embedding safety and sustainability into the design of chemicals and materials. Addressing the "triple crisis" of climate change, biodiversity loss, and pollution, it aligns innovation with societal, safety and environmental goals. However, operationalizing SSbD principles demands rethinking traditional innovation processes to integrate safety and sustainability alongside economic and functional performance, introducing new complexities and uncertainties.

Key challenges include managing trade-offs across safety, environmental, social, and economic dimensions, evaluating long-term impacts, and addressing stakeholder concerns from the design phase onward. While managing uncertainty is inherent in innovation, SSbD introduces novel uncertainties related to sustainability metrics, regulatory requirements requiring systematic and adaptive solutions.

Structured innovation management systems (IMS), such as those outlined in the ISO 56000 can guide alignment with organizational objectives through leadership commitment, strategic direction, operational planning, and continuous improvement. By integrating SSbD principles into an IMS, organizations could potentially foster a culture of innovation that prioritizes safety and sustainability.

The ISO 56000 series outlines eight principles that could support SSbD, for example realizing value, future-focused leadership, adaptability, and managing uncertainty. However, IMS frameworks can be complex, particularly for SMEs. To address this, the study will focus on a simplified model, 4F (Capability for Renewal). 4F focuses on monitoring external trends, managing internal resources, goals with purpose, and the innovation process.

By exploring synergies between ISO 56000 and SSbD, this work offers a first view into practical strategies for embedding safety and sustainability into innovation processes.

6.05.P-Tu456 Balancing the Unseen: Resolving Conflicts in Safe and Sustainable by Design Frameworks

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Contemporary research on Safe and Sustainable by Design (SSbD) approaches faces significant challenges due to a lack of coherent integration of the multifaceted dimensions of sustainability. These gaps create the risk of perpetuating contradictions within sustainability assessments, as the choice and application of indicators often depend on differently oriented conceptualizations of sustainability. To address these challenges, this research investigates conflicting parameter relationships within existing SSbD frameworks. Particular focus is placed on the dynamic interplay between environmental, economic, and social dimensions, aiming to identify areas where trade-offs or conflicting priorities emerge in practice. Such conflicts may result in assessments that overlook critical nuances, limiting their ability to guide sustainable design effectively. By bridging theoretical discourse on sustainability with practical applications, this analysis critically examines the foundations and limitations of current frameworks. Special emphasis is placed on understanding how the relationships between individual parameters such as resource efficiency, economic viability, and social equity affect the aggregation of indicators used in decision-making processes. Understanding these relationships is crucial for designing a robust and reflective SSbD assessment framework. It enables practitioners to anticipate and navigate inherent contradictions while fostering a balanced approach to sustainability that incorporates diverse priorities. Ultimately, this research aims to refine SSbD methodologies, enhancing their ability to guide the development of advanced materials and technologies in ways that genuinely align with sustainability's core.

6.05.P-Tu457 Application of the SSbD Framework in Bio-based Plastics: Lessons Learned from the BIORING Case Studies

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The BIORING project aims to develop a novel portfolio of renewable formulations for UV curable polyurethane biocoatings with optimal biodegradable and/or recyclable features for the construction, furniture, automotive sectors and beyond.

The application of the SSbD faces several challenges, including how to address criteria and data needs at the early stage of innovation. Within the BIORING innovation process, information during the early stages is also scarce, so partners used available foresight tools developed for AdMa to cover information gaps.

In this study we present cost effective approaches and tools applied for an early safety and sustainability assessment of the BIORING materials and processes.

The foresight tools Early4AdMa and LICARA Innovation Scan were used to perform safety, sustainability and cost/benefit assessments to cover steps 1-4 of the SSbD EU Framework at early innovation processes. The selected tools also enabled to identify uncertainties requiring further information later in the innovation process.

Then we conducted a preliminary LCA of the BIORING lab scale processes for an early SSbD step 4 assessment. The inventory was built combining data sources such as information from existing literature, LCA database such as ecoinvent, and experimental results provided by researchers. To reconcile divergences between these different sources, process design and modeling such as ASPEN is a useful digital tool, requiring input from multidisciplinary working group including chemical researchers, LCA experts, and process engineers.

Additionally, with an early-stage process design/modeling we identified bottlenecks in a (chemical) process, e.g. energy intensive steps or built-up of impurities or hazardous chemicals. From a process design, an initial cost estimation was made to initiate an early SSbD step 5 (economic) assessment. This estimation gave insights into what the expensive steps are going to be and where the major improvements could be made. The above-mentioned points are valuable input for R&D, as these provide guidance to which experiments/tests are needed, or where the focus for alternative solutions should lay.

It will be the final aim of BIORING to apply and adapt the SSbD EU Framework to biocoatings.

The Bioring is a Circular Bio-based Europe Joint Undertaking funded project (2023-2026, GA 101112379).

6.05.P-Tu458 Methods and Tools to Assess Exposure and Risk for Steps 2-3 of the SSbD Framework for Advanced Materials

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The European Commission's Safe and Sustainable by Design (SSbD) framework represents a pivotal initiative to integrate safety, environmental sustainability, and societal benefits into the design of materials and chemicals. Advanced Materials (AdMa), including nanomaterials and multicomponent systems, present unique challenges to this framework due to their dynamic properties, life-cycle transformations, and diverse application scenarios. Steps 2 and 3 of the SSbD framework target the critical phases of production, processing, use, and disposal, requiring robust and adaptable tools to assess exposure and risks across these stages.

This study systematically reviewed 31 tools for exposure and risk assessment, categorizing them into three tiers based on complexity, data requirements, and usability. The review identified gaps in tool availability, with some tools outdated or inaccessible, and highlighted promising advancements from recent projects, although some are not yet widely available to the public. Additionally, challenges persist in addressing AdMa-specific behaviours, including aggregation, dissolution, and transformations throughout their life-cycle.

The findings underscore three critical challenges: persistent data gaps, the need for better integration of

tools across life-cycle stages, and the limited accessibility of high-complexity tools for smaller enterprises. Overcoming these obstacles will require the development of adaptable methodologies, enhanced data-sharing practices, and cross-sector collaboration to make tools more user-friendly and broadly applicable. This study provides a comprehensive assessment of tool readiness and highlights actionable insights to support the effective implementation of SSbD principles for AdMa, paving the way for safer and more sustainable innovation.

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6.05.P-Tu459 Integrating Computational Models and Balancing SSbD Dimensions to Satisfy the Industry Needs for Responsible Innovation on Advanced Materials and Chemicals

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Safe-and-Sustainable-by-Design (SSbD) advanced materials and chemicals (AMC) are a central requirement for reaching the ambitious goal of making Europe the first digitally enabled circular, climate-neutral, and sustainable economy. Novel AMC need to provide the high functionality required for their advanced applications, whilst simultaneously exhibiting improved safety and sustainability performance considering the complete value chain and life cycle. To facilitate adoption by industry and, by doing so, foster the twin green and digital transition of Europe's economy, the PINK Project aims to produce innovative modelling software and integrated workflows for the development of AMCs, which are combined into an industry-ready open innovation platform, the PINK In Silico Hub. PINK takes a holistic by-Design approach targeting the primary goal to satisfy the needs of industry, as implementing SSbD presents the multi-objective optimization problem to balance the four requirement categories functionality, cost-efficiency, safety, and sustainability. PINK employs industry-driven developmental Case Studies establishing scenarios that operate at different development stages within the industrial AMC innovation pipeline: Scenario 1 at business opportunity stage where multiple candidates can be generated in silico and potentially later arising sustainability constraints are to be determined by predictive modelling in parallel to functional performance testing; Scenario 2 at innovation development stage defined by one lead structure identified and some information is available with in silico tool-based modifications going on and sustainability criteria being evaluated; Scenario 3 at later material design stages where more data for several dimensions becomes available. Computational models are assembled in the PINK Matrix that enable an automated SSbD guidance prioritized according to industry-relevant implementation profiles. Depending on case requirements and scenarios envisioned, PINK intends to address elements of all five steps of the SSbD Framework, employing selected in silico tools, continuously improving the confidence in the predictions over multiple design cycles by producing new knowledge on a constantly reduced set of better performing candidates for replacing substances of concern. Industry readiness shall be guaranteed by improving usability, practicability, user experience, data provenance, documentation, and security.

6.05.P-Tu460 Navigating through Safe-and-Sustainable-by-Design (SSbD): Scoping Analysis

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Safe-and-sustainable-by-design (SSbD) is an integral component of the European Chemicals Strategy for Sustainability (CSS) action plan which aims to reduce negative impacts on human health and the environment associated with chemicals, materials, products and services commercialized or introduced onto the EU market (EC 2020). This has led to a series of publications from the European Commission (EC) Joint Research Centre (JRC) including: i) a review of safety and sustainability dimensions, aspects, methods, indicators and tools (Caldeira, Farcal et al. 2022); ii) the SSbD framework for the definition of criteria and evaluation procedure for chemicals (Caldeira, Farcal et al. 2022); iii) the application of SSbD framework to case studies (Caldeira, Garmendia Aguirre et al. 2023); and iv) the SSbD chemicals and materials methodological guidance (Abbate, Garmendia Aguirre et al. 2024). The aim of the SSbD framework is to support the design and development of safe and sustainable life cycles of chemicals and materials with research and innovation (R&I) activities. The recently published methodological guidance introduces a scoping analysis which supports the contextualization of the assessment phase of the SSbD framework in R&I activities (Abbate, Garmendia Aguirre et al. 2024). The Scoping Analysis builds on: i) the system definition, ii) the (re)design definition, iii) the definition of the system boundaries and iv) engagement with the actors along the life cycle. These three building blocks are necessary, but they can be

implemented in a different order based on the industrial cases. Here a case study is presented on the application of the scoping analysis. Commercial flame retardants for polymers are currently the standard materials used in the automotive industry; however, they pose issues related to high persistence and allergenic potential. In this context, graphene oxide presents itself as a versatile material, as it can be functionalized with different additives of biological or natural origin to improve its properties and reduce the toxicity. SSbD strategies were generated for a graphene oxide functionalized with chitosan (N-acetylglucosamine), a benign biopolymer with flame retardant properties used as a substitute for halogenated flame-retardant products. This option does not use hazardous materials, reduces consumption of water in production process, reuses material lost during production and minimizes occupational exposure.

6.05.P-Tu461 Interoperability fir bridging SSbD Dimensions, Value Chains and Material Life Cycle Stages

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Industry is in need of advanced materials and chemicals to comply with the goals of the European Green Deal based on a twin green and digital transformation. These must provide the expected new or improved functionality and, at the same time, be both safe and sustainable, i.e. need to be developed following the Safe-and-Sustainable-by-Design (SSbD) paradigm.

Many projects including PINK and MACRAMÉ are developing new experimental and computational methods supporting the design of SSbD materials considering the complexity of the materials themselves as well as the new challenges resulting from the need to characterize the materials and products, they are used in, at the use and end-of-life stages of the material life cycle. Being able to apply all these tools in real-world scenarios and, in this way, supporting industry to adopt a SSbD mentality is not only a question of availability of tools. They also need to be interoperable, trustworthy, and future proof, i.e. tools from different providers have to work together, results must be understandable to allow informed decisions and today s tools need to be complemented or even replaced by new tools for improving accuracy and confidence.

We are currently designing and implementing major components for a technical and semantic Interoperability Framework. This is based, on the one hand, on microservices providing the data resource, model and software and, on the other hand, on data documentation and annotation on different levels based on clearly defined data models, combination and harmonization of chemical, material, biological and medical ontologies as well as ontologies to annotate (meta)data models. On top of this framework, multiple infrastructure components are built: (i) the Project Registries (e.g. MACRAMÉ, BIO-SUSHY and PINK), which index all available services and data, (ii) workflows combining different services, (iii) an integrated knowledge graphs construction in form of the PINK Knowledge Base (PINK KB) providing relationships between tools, and (iv) the components of a central PINKISH platform (decision support workflow, generative AI for proposing new materials and a chemical/material dashboard). The presentation will describe these new concepts, approaches and tools and how data and models will, as a consequence of more general adoption, become more independent so that they can be sustained even if the original databases or platforms (including PINKISH) are retired.

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6.05.P-Tu462 Bringing Safe-and-Sustainable-by-Design to the Public – Exploring Stakeholders' Concerns and Needs Towards Nanotechnologies

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Nanomaterials are characterised by their great potential in research and industry, but at the same time are still relatively little known to the public. Even though many application areas of nanomaterials, including

cosmetics, medicine and electronics, are advancing to provide a more Safe-and-Sustainable-by-Design (SSbD) approach, public perceptions towards nanotechnology are sceptical. Within this study, a multi-method approach was used in order to examine relevant factors on public and stakeholders perceptions. Firstly, a multi-level media analysis in Germany observed the societal discourse on nanomaterials, different actor groups and factors of public acceptance. This analysis included data from newspaper articles (N=272), semi-scientific magazine articles (N=99) and on the social media level user comments from thirteen YouTube videos on nanotechnology advancements. Following, expert interviews and a pedestrian survey in Germany (N=34) were conducted to prepare an expert workshop (N=20) to discuss the potential and challenges of public trust towards nanomaterials in sunscreens. In addition, a survey along stakeholders in nanotechnologies was conducted.

Findings from the media analysis showed that the reporting of technology development was predominantly descriptive and did not discuss the social implications for the public. There was an ambiguity concerning the health and ecological risks of nanomaterials in the articles, which portrays the scientific uncertainty of this technology. Furthermore, social media users were opinionated towards nanotechnology, ranging from sceptical views of potential misuse to hopeful and curious outlooks on the future of applications. There tends to be disappointment in not receiving the promised advancements in nanoscience, shaped by its intangible nature and existing associations of the technology. Thus, there is a need for continued efforts to enhance public engagement by increasing and diversifying the range of topics in news reports and creating outreach activities from social media channels to disseminate information. Based on the results and under the motto Bringing SSbD to the public, information and communication materials such as factsheets and a nano-quiz were developed and distributed, thereby gaining important experience in how people can be reached and addressed on this complex topic through science communication.

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6.06 Communication for Tomorrow: From Experimental Design Towards Societal Impact

6.06.T-01 Bridging Science and Law: Effective Communication in Water Reuse Projects

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Climate change and environmental researchers are united by a shared understanding of the urgency of the environmental problems our world is facing. However, what often separates them is a shared vocabulary and language. Depending on their academic background, each scholar will interpret and use concepts such as climate mitigation, ecosystem or water regulation according to the customs in their scientific discipline. Often falsely assuming others have the same understanding as them of these central concepts. These potential misconceptions can hinder to practically and effectively communicate research findings and propose solutions that ultimately lead to environmental and, therefore, societal benefits.

This work draws on insights gained from the interdisciplinary research project AquaConnect, which focuses on transitioning the Dutch water management system toward circularity, where water is reused instead of discharged. Workshops held throughout the project offered open communication and discussions to align the research questions towards stakeholders needs. These discussions highlighted the need for a harmonized vocabulary across disciplines in water reuse research, especially the science-law relationship. Differing interpretations of key phrase for water reuse between legal frameworks and scientific literature show that this can lead to legal grey areas. Such situations offer room for practitioners to adapt innovative solutions to environmental problems but simultaneously deter practitioners due to unclear regulatory boundaries.

This work emphasises the need to include legal scholars early on in interdisciplinary work which focuses on environmental problems that are influenced by regulatory frameworks. Using water reuse as a case example shows that at the intersection of human and environmental health, multiple legal as well as scientific perspectives must be recognised and defined to propose effective solutions. Therefore,

addressing legal implications alongside scientific practises and stakeholder engagement, allows research projects to be shaped towards deliverables that can effectively benefit the environment and society.

6.06.T-02 What's in Our Water? Sailor's Scientific Strategies

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Societal interest in water quality has increased whilst water quality has been reported to decrease in the UK over recent years. The decline in water quality is often attributed to a combination of increased sewage overflows, changes of land use, plastic mismanagement and introduction of new chemicals and materials into circulation. Young people are also often reported to be one of the groups that are most impacted by eco-anxiety, which is a term that is used to describe the hopelessness and frustration often felt by people when they are faced with environmental issues.

Through this project, we worked with Sea Cadets UK, a charity working with young people aged 9-18 in the UK with a focus of maritime activities. The projects had a two-step approach to firstly; develop a practical workshop exploring data collection and advocacy and secondly; a sampling campaign to collect water quality data in environments that they use for boating. The aim of the project was to involve people in those environments with the experimental design and data collection for these sites, and to co-create a workshop to share this process and the results. Establishing the common ground of water quality assessment and the challenges with balancing different societal and environmental pressures for management plans also facilitated the further development of discussion on Advanced Materials risks and ecotoxicity methods used to assess these.

Testing included temperature, pH and conductivity, nitrates and phosphates and a secchi disk to test the turbidity. Sampling took place at 8 sites across the UK including a mix of coastal, freshwater, brackish, estuarine and Sites of Special Scientific Interest.. Partnership building was an important aspect in this project and the interdisciplinary expertise across the network was highlighted, spanning pedagogy, photography and social media which added value to the project and outputs that could not have been achieved by a dissemination model from the research team.

In parallel, a practical workshop (230 attendees) was designed with the Sea Cadets Learning and Development team to facilitate discussion on environmental advocacy, the need for robust data and evidence and how to engage with decision makers (i.e. policy) going forwards. The workshop also included analysis of microplastics in water samples, which lead to discussion on analytical method development for particles, the challenges and the ecotoxicity risks and methods for freshwaters.

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6.06.T-03 The Price of PFAS: How Access to PFAS Information Affects Willingness-to-Pay for Products Labelled as “PFAS Free” or “Contains PFAS”

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Consumers are already faced with products labelled as Per- and poly-fluoroalkyl (PFAS) free. Meanwhile, regulation of some PFAS means products containing PFAS could soon carry a warning label. Yet public knowledge of PFAS remains low in Europe. How do consumers perceive such labels, and how does knowledge about PFAS affect it? We tested how people perceive free-from vs. warning labels on shoes with and without having information on PFAS provided. Based on other work, we predicted that participants given information would have higher WTP for products labelled as PFAS free and lower WTP for products labelled as Contains PFAS, compared to those who did not receive information. We also measured how emotional affect influenced decision-making, as prior work has shown that consumers tend to make decisions which reduce negative feelings and promote positive ones. To test our hypotheses, 406 participants were shown shoes and were asked how much they would pay for the product, and about the emotions they felt when viewing the product. Participants first saw shoes with no labels (i.e., baseline), and then with PFAS free and Contains PFAS labels in randomized order. The PFAS free label featured a leaf motif alongside the text. The Contains PFAS label featured pictograms with a red hazard diamond and text denoting persistent, mobile and toxic properties. Half of participants rated the shoes having been given further information on PFAS, including examples of function, risks, benefits and regulation of PFAS. Analysis used linear-mixed effects models. Since 93 participants reported having some knowledge of PFAS, we also included prior PFAS knowledge as a predictor. The PFAS free label led to significantly greater WTP than did the Contains PFAS label. Positive affect led to higher WTP and vice-versa for negative affect. Being given PFAS information during the study increased WTP for the PFAS-free label,

as did having prior knowledge on PFAS. What is crucial is the role of both information about PFAS (whether known prior or provided during the study) and emotions in mediating labelling effects. Having more information increased willingness-to-pay to avoid products with PFAS. Affect was significantly tied to consumer intention, and positive emotions were a stronger predictor than negative ones. Such data can provide insights on effective communications with consumers, particularly by accounting for psychological factors affecting perception.

Disclaimer/Disclosure: This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant agreement No 101036756, project ZeroPM: Zero pollution of persistent, mobile substances.

6.06.T-04 Cosmetics Labelling and Ingredients: Disentangling False-Claims and Empowering Consumers

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I find information for the general public (not research publications) very confusing and scary. As a reasonably informed consumer, and a PhD student exploring the regulatory landscape for chemicals and their applications in cosmetics and personal care products, the more I read the more confused I get! So how on earth can the general public know what is safe and what is not? What is a genuine claim of sustainably sourced, natural or cruelty-free versus a green-washing statement that hides a multitude of murky practice under mis-information? When do consumers genuinely need to be concerned that there are harmful chemicals in their cosmetics, versus understanding that a lack of data to confirm the absence of harm underpins the precautionary principle, versus some data showing harm but at concentrations far above those that a consumer would ever be exposed to and not via a relevant exposure route?

Adding to the public communication challenges is (1) the breadth of regulations / directives that converge in the cosmetics area; (2) the complexity of ingredients lists and lack of clarity regarding the role of each ingredient in the product and that only ingredients above 1 wt% need to be reported; (3) the lack of data to rule out harm being conflated as evidence of harm (even by experts and regulators); (4) the massive exposure of the population estimates indicate that a typical product contains anything from 15-50 ingredients, and the average adult uses 9-15 personal care products per day, resulting in adults placing around 515 individual chemicals on their skin daily via cosmetics; and (5) the fact that the beauty industry is rife with illegal imports that evade EU regulation and mis-use of toxins (Botox) and medical substances (Ozempic) which are being mis-used.

In this work, we tease out these questions, by assessing the regulatory and voluntary certification landscape for cosmetics, the available tools and resources for evaluating the chemicals in cosmetics products (voluntary reporting schemes, mobile phone apps that scan product labels and report on their overall safety profile and generate case studies to communicate the key issues and a decision tree for identification of safe, sustainable and green cosmetics. One case study considered is the question of whether naturally occurring is always safer (better) than synthetic via a comparison of natural versus synthetic mica as part of the transition to biodegradable glitters.

6.06.T-05 Getting Beyond the Bubble: Measuring Effectiveness of Outreach Activities for SSbD-Related EU Projects

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Effective scientific communication is vital for enhancing the societal impact of research, particularly in the context of projects funded by the European Commission's Horizon Europe program. Despite its importance, science communication often relies on a combination of intuition and empirical evidence, especially when addressing abstract or complex topics like Safe-and-Sustainable-by-Design (SSbD) principles. This raises critical questions about how to effectively engage audiences and foster the adoption of new scientific concepts.

This study explores strategies for maximizing the outreach and impact of science communication efforts, focusing on both digital platforms and in-person activities. The primary digital platform of interest is LinkedIn, a tool for engaging diverse professional audiences. Engagement metrics such as clicks, reactions, comments, and shares are analyzed from the LinkedIn profiles of ten EU-funded projects over a one-year period. Special attention is given to the types of posts that generate the highest levels of engagement and their reach beyond the immediate scientific community. Demographic analysis of the interacting audiences, combined with project's website traffic data, provides insights into how different audiences discover and interact with scientific content.

In parallel, the effectiveness of in-person engagement activities, including workshops and public science events, is assessed to compare their reach and impact with online methods. These events, though less quantifiable, provide invaluable qualitative insights into stakeholder engagement.

The findings from this study aim to identify best practices for designing science communication strategies that effectively engage a broader audience. Practical recommendations will be provided to enhance communication and dissemination efforts, ultimately contributing to the successful adoption of complex scientific principles across various stakeholders and sectors.

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6.06.P Communication for Tomorrow: From Experimental Design Towards Societal Impact

6.06.P-We499 Using Art to Expand Science Understanding in the Context of River Health

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Over 30 years, and across 26 reports, the Richmond River, New South Wales, Australia, has consistently been classified as stressed and degraded with the catchment in worse ecological health than most estuaries in NSW. Drainage of wetlands in the 1800s and 1900s for flood mitigation and to support the development of agriculture has caused significant acid-sulphate soil problems. The depletion of riparian zones and nutrient runoff from agriculture and horticulture have caused excessive sedimentation and replacement of water tolerant vegetation with pasture grasses has led to flood related low oxygen levels and fish kills.

Similar degradation of rivers and wetlands across Australia is linked to long-term controversy and conflict between Indigenous peoples, environmental managers, irrigators and farmers, fishers, local councils, and State and Federal governments, with the core of the conflict being the work that rivers are asked to perform for very different groups of people. If Australia is to improve its relationship with and use of rivers then more holistic ways of understanding rivers, and strategies for representing and communicating this understanding, must be developed and brought together. Communicating the science of river health can be supported by using multi-disciplinary and innovative approaches.

During weekly water quality sampling for some 16 months a 250mL water sample was collected from each site for a dedicated artistic focus which evolved through practice led open-ended enquiry. Each 250mL sample was filtered and the filter paper retained. The tonal and highly varied visual samples provided the foundation of a large 11m exhibit that linked to water quality data and over 100 sediment samples collected from across the whole 22 446 km² catchment. The exhibit was presented in the Northern Rivers Community Gallery as part of a larger exhibition *Speaking with the River* and included participatory workshops. The workshops engaged broader expertise to explore river health using our 5 senses and much more followed.

6.06.P-We500 Comic Strips as a Science Communication Tool

Serena George, University of Wisconsin-Madison, United States

My interaction with one perceptive 11-year-old sums up why laboratory animal research is so controversial. After I explained my toxicology research, which measures fish survival upon exposure to a chemical, she asked, So, you're becoming a veterinarian to save animals, but you're killing them?

Researchers must constantly re-evaluate animal welfare principles and the ethics of sacrificing the few to save the many. At the same time, the relevance of highly controlled laboratory studies is aptly questioned by, for example, people whose livelihoods depend on production of these chemicals and the scientists who are trying to regulate them. I want to address this question I keep hearing: Why does laboratory research matter? Through a comic strip created with digitized ink and wash drawings, I tell the story of a laboratory fish who wonders what life is like beyond the walls of his tank. The character reflects on the sense of duty he feels toward keeping his wild friends safe through his participation in laboratory research; he considers the welfare benefits that life in the laboratory affords him; and he questions the real-world applications of studies conducted in such a controlled and simplified laboratory environment. When he is suddenly scooped up by a researcher and placed in an experiment in a real Wisconsin lake, he experiences the adventure of life in the wild and comes to appreciate his home in the laboratory. Our scaly, underwater friends tucked away in a laboratory can sometimes seem unrelatable. Through storytelling, art, and

experimental data explained in plain language, this comic strip encourages the reader to view science from a new and hopefully more accessible perspective. The reader is invited to experience the excitement of scientific discovery and appreciate how laboratory research affects their everyday life. Other scientists may hopefully find inspiration on how to share their science story in a creative way to reach a broader audience.

6.06.P-We501 Knowledge, Attitudes, and Practices Towards Plastic Clean-up Technologies

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In recent years, innovators have developed various technologies to facilitate the removal of mismanaged plastic, a growing global issue associated with poor management during the different stages of plastic's life cycle. These technologies may be a beneficial resource for engaging public awareness, providing plastic pollution data, and collecting and removing plastic from the environment. However, due to the novelty of these technologies, the relevant knowledge, attitudes, and practices regarding these technologies remain unclear. Using a mixed-method approach, the present study investigated the general public and stakeholders' perceptions of plastic pollution, their knowledge and attitudes towards plastic clean-up technologies, and current practices. Cross-sectional survey data was collected from a representative sample of 948 adults living in Belgium, while qualitative interview data was gathered from seven stakeholders associated with areas of potential deployment of plastic clean-up technologies. Our preliminary descriptive results showed that the majority of participants considered plastic pollution to be a very big problem, and 46.9 % had heard of "plastic clean-up technologies" before, but only 6.6 % had ever seen one deployed in real life. Over 40% of the participants indicated having no knowledge at all about plastic clean-up technologies. Despite this, 84.2% agreed that plastic clean-up technologies can have a long-term positive impact on the environment in which they are deployed, and a percentage of 1.7 % disagreed. Further results will present associations between individual factors (sociodemographic, techno-optimism, value orientation) and the knowledge and attitudes toward plastic clean-up technologies. The interview data from the stakeholders will be analyzed by the time of the conference. In light of the fifth session of the Intergovernmental Negotiating Committee (INC-5) for an international and legally binding treaty to reduce plastic pollution, our preliminary results indicate that the public generally thinks that plastic clean-up technologies could lead to improvement in the area of deployment over time.

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6.06.P-We502 Safety and Sustainability of Innovative Materials – The Communication and Knowledge Base MANTRA

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There is a great need for new and innovative materials in order to adequately meet the numerous societal challenges of our time. Some non-exhaustive examples of this are materials to secure the green energy transition (e.g. in production of green hydrogen) or materials replacing or reducing the use of rare elements (e.g. in industrial catalysts for processes that require little energy). Adaptation to climate change or securing clean water resources are other areas with a need for newly developed materials. However, not only the effectiveness and efficiency of the materials for the applications must be considered, but also the safety for humans and the environment as well as sustainability must be ensured.

The project MANTRA - Data on innovative materials for sustainability and transfer has the important task of supporting research projects that are developing promising materials and processes specifically for the use in membranes and catalysis. Support is provided in science communication, networking, public relations, development of sustainability indicators, advice on materials safety and industry and practice transfer.

In addition, MANTRA strives to present knowledge about health effects and sustainability of new materials and processes in the field of catalysis and membranes to the public via a website (<https://materialneutral.info/en/>), brochures and information flyers. Furthermore, status meetings, workshops and involvement in conferences facilitate the transfer of knowledge on material safety and sustainability between the relevant stakeholders, from material developers to consumers.

Specific challenges of the project include (1) data scarcity on materials safety for rare materials, (2) the almost unmanageable amount of data for common elements (e.g. TiO₂), and (3) the allocation and selection of sustainability indicators from existing frameworks that are applicable to novel materials, addressing all three dimensions of sustainability. The project will develop strategies to overcome these challenges.

This poster is designed to present the MANTRA knowledge base, the scientists behind the project, and the goals and methods in communicating the safety and sustainability of innovative materials.

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6.06.P-We503 Building Networks for Outreach and Impact

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Background Swedish Life Cycle Center

The Swedish Life Cycle Center, established in 1996 and hosted by Chalmers University of Technology, is a center of excellence that fosters collaboration among academia, research institutes, industry, and government agencies. The Center's vision is to accelerate sustainable transition through science-based life cycle action. The Center is a recognized player within the field with multidisciplinary methodology and collaboration between researchers, practitioners and decision makers. Operating as a neutral platform, the Center allows for mutual project development and co-creation among researchers, practitioners, and decision-makers. This neutrality is supported by a strong academia base, ensuring scientific credibility and transparency. A key part of our mission is to expand the understanding and application of the life cycle perspective. The Center is dedicated to developing communication efforts that make advancements in the field more accessible and actionable for a wider range of stakeholders.

Methods for Outreach

Network Building: Initially focused on large industrial companies and academia, the Center has expanded its network to include authorities and SMEs. Events and projects invite diverse participation, fostering influence and action. Networking remains a key outreach method.

Innovation Cluster: Funded by the Swedish Energy Agency and partners, projects aimed at wider audiences have both developed new digital communication channels and strengthened the network. Newsletters, LinkedIn, and YouTube channels have seen significant growth, with a tenfold increase on LinkedIn over five years.

Webinar Concept: In 2022, the Center launched "Life Cycle Talks," a webinar series presenting the research front to keep you up to date with the science and application of the life cycle perspective. This concept has built a platform for sharing, discussing, and exploring the latest research among researchers, government agencies, and industry while also driving new subscribers to the Swedish Life Cycle Center YouTube channel.

Summary

Grounded in its core values, network, and digital communication channels, the Center has significantly influenced decision-makers in industry and policy, promoting life cycle action. In the presentation we will share our methods and concepts which are transferable to any field requiring competence building, collaboration, and action-oriented decision-making

6.06.P-We504 Persistently Negative? Consumer Responses to Warning Labels Denoting Persistence, Mobility and Toxicity of Chemicals in Everyday Items

Nina Vaupotic¹, Jules Schlosser¹, Sarah Hale², Sabine Pahl¹, Mathew White¹ and Ellise Marissa Suffill¹, (1)University of Vienna, Austria, (2)DVGW-Technologiezentrum Wasser, Germany

Assessing risk of substances is an important part of managing chemicals, but risk assessment procedures may not be understood by the public who instead express risk perception. Emotional affect, concern and support for regulation are important for understanding risk perception. One way of communicating risk is

via labels that indicate specific chemical characteristics, e.g., to consumers. These labels may carry warnings for persistence (P), mobility (M) and toxicity (T) in the case of some per- and poly-fluoroalkyls (PFAS). Yet studies so far tend to measure chemical risk perception only very broadly, e.g., by describing the public's chemophobia, a general negative disposition towards chemicals. More research is needed on how non-experts perceive specific characteristics on product labels, because consumer understanding and behaviour is one route to reducing use. This study is novel in testing risk perception of labels for combinations of P, M and T in everyday products. We predicted toxicity would produce the largest increase in risk perception, compared with persistence and mobility, due to greater non-expert familiarity with toxicity. We also examined whether differences in likely body contact with the product would increase risk perception based on prior work. 321 participants saw one of two products at baseline (no warnings) and rated them for affect, concern about using the product, and support for regulation of chemicals in the product. Participants then saw and rated the same kind of product another seven times with warning labels showing different combinations of characteristics in randomized order. Results were analysed with linear-mixed effects models. While we confirmed toxicity had the largest effects of any single characteristic (i.e., more negative affect, increased concern & support for regulation), persistence and mobility also showed these patterns over baseline (albeit to a lesser extent). Products with all three chemical characteristics (i.e., PMT) were seen as the riskiest. Greater likelihood of body contact increased negative affect. Despite differences in risk assessment vs. perception, results suggest that non-experts are somewhat sensitive to risks posed by PM substances (not only toxicity), and in general highly support chemical regulation of consumer products. We argue such data can inform better communication about chemicals, improve understanding of warning labels and bolster consumer support in reducing PFAS use.

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6.06.P-We505 Estuarine & Marine Ecological Risk Assessment Research Immersion Model Improves Science Literacy and Student Engagement in Local and International Environmental Issues

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The Tijuana River Estuary (TRE), the largest coastal wetland in Southern California has been a source of contention between the United States and Mexico for decades due to transboundary pollution. The Boz Life Science Research and Teaching Institute and University of California Division of Extended Studies piloted research immersion programs for pre-college students to conduct environmental health assessments. Sediment and water samples from four sites during dry and wet seasons were used in sea urchin embryotoxicity testing; the abundance of sediment-bound chemical pollutants was investigated by qualitative non-target analysis and quantitative targeted analysis using GCMS and LC MS/MS. Novel composite integrative samplers (CIPS) were deployed in water to quantify polar and non-polar chemicals, providing time-weighted-average concentrations. 16S rRNA sequencing was used to explore correlations between microbial diversity and chemical contamination. Pre-college students interpreted scientific literature, generated hypotheses, designed and executed experiments, applied biostatistics and bioinformatics to analyze data and infer biological relevance. The program concluded with a student presentation during a symposium attended by scientists, educators, government officials, and industry leaders. A similar method was applied to promote the engagement of high school students at the Croatian island of Hvar: to assess the potential adverse anthropogenic effects on sea water quality, a comprehensive ecological risk assessment was initiated. CIPS were deployed for ten days at 16 sites covering both low and high levels of human activities along the 65 km island, which is one of the most popular summer tourist destinations in the world and a UNESCO heritage site. Students also collected native mussels (*Mytilus galloprovincialis*) and sea urchin (*Arbacia lixula*) at these sites to correlate body burdens with CIPS chemistry profiles; animal tissues will be used for metagenomic analysis. Animal tissues will also be used for gene expression profiling to identify biomarkers of exposure to complex industrial chemical mixtures. Besides identifying public health hazards and promoting legislative action, the goal for both programs is to serve as foundations for a long-term international research collaborative and students exchange program focused on pressing environmental issues while cultivating future STEM community leaders and stewards of responsible environmental policies.

Disclaimer/Disclosure: The Tijuana River Estuary Research Immersion Program is supported by the University of California San Diego, Division of Extended Studies in partnership with The Inamori Foundation and Girard Foundation as part of the development and implementation of Futures Life Sciences student research immersion program at the Boz Life Science Research and Teaching Institute.

The Island of Hvar Ecological Research Assessment Pilot Project has been generously supported by the Matko and Vera Mili?i? Foundation, Mayor's offices from Hvar and Stari Grad, and the Island of Hvar Nautical Center. Funding for research is provided by Statera, Inc (North Carolina, U.S.), and Boz Life Science Institute (San Diego, U.S.).

6.06.P-We506 Mainstreaming the Fish Invitrome for Animal-free Environmental Risk Assessment: An Expert Interview Campaign

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Large numbers of fish are being used each year for chemical risk assessment, raising ethical, practical, and economic concerns. Given the need to reduce animal testing, new approach methods (NAMs) using fish cell lines have been developed over the past decades. To strengthen confidence in the predictions provided by these fish cell line-based assays, we aim to develop a modular framework, the fish invitrome, capable of predicting chemical toxicity without the use of animals. This framework comprises several modules to assess different effects, such as mortality (using the RTgill-W1 cell line assay (OECD TG 249)), chemical bioaccumulation/biotransformation, inhibition of growth, neurotoxicity, and reproductive toxicity.

Establishing a new method and achieving the status of a validated method, such as an OECD test guideline (TG), takes at least a decade, similar to developing a new pharmaceutical. Still, even after the adoption as an OECD TG 249 in 2021, the global status of implementation of the RTgill-W1 cell line assay in risk assessment practices remains unclear. Therefore, to accelerate the uptake of that assay and of the expanded fish invitrome framework, our objective is to identify different pathways of application in specific ecotoxicological application contexts, rather than focusing solely on immediate regulatory acceptance. This requires a deeper understanding of current ecotoxicology risk assessment practices by different regulatory agencies, industries, NGOs, and researchers. As a first step, therefore, we performed an expert interview campaign with a dozen representatives from different industrial sectors to understand (1) what kind of risk assessment is being done, (2) how fish cell line tests could fulfil their ecotoxicology assessment needs, and (3) how the field of ecotoxicological assessment might develop over the coming years.

This interview campaign highlighted different challenges and barriers across the industrial sectors in the implementation of these NAMs. These results will allow us to develop a stepwise approach for mainstreaming the fish invitrome framework, in order to make it widely understood, trusted, and used in various ecotoxicological applications. This socio-technical approach promises to promote a broader use of the fish invitrome framework in practical applications while gradually overcoming institutional, cultural, and technical barriers.

6.06.P-We507 Dear Dearbhla: Nature's Agony Aunt

Katie Reilly and **Iseult Lynch**, *University of Birmingham, United Kingdom*

Dear Deabhla much like an agony aunt gives life advice, we can turn to indicator species, and model organisms for environmental advice. What will happen if temperatures rise in freshwater ponds? How might microplastics change algae populations in rivers? *Daphnia*, or waterfleas, have been recognised as sentient species in freshwater environments for a long time, and are widely used as a proxy, or model, species to understand how environmental challenges might affect ecosystems. Dearbhla, the *Daphnia*, takes a novel approach to science communication by taking the model organism, bringing them out of the laboratory and anthropomorphising them, quite literally in the form of a *Daphnia* costume. This allows us to highlight the scientific importance and development of *Daphnia*, or water fleas, as an amazing model organism that are used globally as indicators of good environmental health. They have been historically used for freshwater toxicity testing of chemicals, and more recently nanomaterials and microplastics. For context, *Daphnia* are transparent organism and are keystone species in freshwater environments because they link the primary producers (algae) with higher trophic levels in the food web (fish and other insects etc). Under healthy/normal conditions they will reproduce genetically identical daughter offspring and will only produce male *Daphnia* when stressed, which is reflected in Dearbhla having a removable brood

pouch. A range of methods that have been developed to monitor environmental health such as the use of fluorescent particles and dyes, video capture methods to explore how their movement changes and a range of molecular methods to determine their cellular response to pollutants. Dearbhla can bring all of this research to the public in an engaging and visual way and supports some of the hands-on activities. Some of these events have included science festivals and open day events where Dearbhla has attended, and acted as an agony aunt to answer the public questions on environments issues. We have found this to be really engaging due to the visual nature, the opportunity to take photos and the novel element - many people haven't seen a Daphnia so having a human sized one draws some interest! Dearbhla creates a novel lens through which to engage different audiences with new environmental science and ecotoxicological approaches, which allowing two-way communication between model organisms and the public.

6.07 Environmental Risk Assessment Under Biocides and Other EU Legislations

6.07.T-01 Take EU Biocides Legislation to the Next Level

Stefanie Wieck, Corinna Burkart and Sascha Setzer, German Environment Agency (UBA), Germany
Biocides are chemicals or microorganisms used to control harmful organisms to protect human or animal health or materials, but their use can pose environmental risks. The European Union's Biocidal Products Regulation (EU) No 528/2012 (BPR) governs approval and authorisation, requiring environmental risk assessments (ERA) for both active substances and products in a two-step process. It involves hazard-based and risk-based assessments.

Since entry-into-force of the BPR in 2013, there have been many lessons learned from the implementation of the regulation. Additionally, the One Substance One Assessment (OSOA) approach was introduced by EU in 2020. Based on the experiences made, research projects and the new OSOA approach, the authors compiled recommendations to make BPR fit for the future. This contribution aims to provide recommendations to enhance environmental protection and accelerate ERA procedures.

Enhancing Environmental Protection: Gaps in BPR might lead to preventable environmental risks, particularly in the areas of endocrine disruption, simplified authorisation processes, and the sustainable use of biocidal products. Proposals include strengthening the requirements for simplified authorisations, and incorporating principles for sustainable use during the application phase. These changes could help minimize environmental harm, improve public communication, and support sustainable management of harmful organisms.

Accelerating Risk Assessment Processes: Delays in the approval of active substances and product authorisations can be driven by the complexity of ERA and ambiguous regulatory language. The authors suggest pragmatic approaches to streamline the assessment of disinfection by-products and address issues arising from substances regulated under multiple chemical legislations.

BPR has been a milestone towards a better protection of the environment. However, in times of Zero Pollution Ambition, OSOA and more than a decade of experiences with the legislation, it is time to take it to the next level.

6.07.T-02 A City is Not a Field - Progress in the Groundwater Assessment for Urban Biocide Emissions

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The environmental risk of emissions of biocides from construction materials in urban areas is well documented. Precipitation can lead to significant entries of biocides and their transformation products into surface waters and groundwater aquifers. Emission scenarios are established for the modelling of biocide releases to surface water in cities. However, a model taking into account the specific conditions in urban soils and entry pathways to groundwater is currently not harmonised for the regulation of biocidal products.

We developed realistic worst-case scenarios in order to achieve a more realistic assessment of biocide emissions from building façades into urban groundwater. Thereby, urban soil properties and depths to groundwater were thoroughly analysed and considered. Representative entry pathways in urban areas namely vegetated soils, permeable pavements and infiltration systems - were integrated as well as a refined building geometry and a realistic building density for central Europe. National technical guidelines on the design of swales and permeable pavements in Europe were analysed to identify further key parameters. These input data were integrated into the dynamic leaching model COMLEAM (CONstruction Material LEAching Model) and the soil transport models PEARL and PELMO. By including all three

emission pathways to the model chain, biocidal emissions to urban groundwater could more realistically be described.

Based on the gained knowledge we elaborated a guideline for architects, engineers, and municipalities. Hereby, sustainable construction practices shall be enhanced to reduce biocide emissions in urban areas. This can lead to better groundwater protection and more sustainable rainwater management in modern blue-green infrastructure strategies.

In the next step, the existing Emission Scenario Documents (ESD) for biocides need to be adapted for an adequate consideration of the outcome in regulatory processes. Recommendations for a more realistic exposure assessment are presented, specifically for the characterisation of building geometries and the inclusion of the diverse urban emission pathways.

6.07.T-03 Comparison of Environmental Exposure Assessment Strategies in Different EU Legislation

Heike Schimmelpfennig, Brill Regulatory Services GmbH, Germany

Introduction: Before chemical substances and/or products are placed on the EU market, their risk to human health and to the environment has to be assessed according to the intended use. Depending on their use, substances and/or products are regulated under different EU legislation. In the case of dual use (e.g. disinfectants used as medical device and biocide), the same substances and/or products are assessed under different legislation.

This abstract compares and evaluates the different and common ways of performing an environmental exposure assessment in different legislation. The focus is on similarities and differences and it is assessed whether they can be harmonised.

Materials and Methods: The first step is to identify legal areas where the same substance is used under different EU directives or regulations (e.g. insecticides used in stables as biocide or veterinary products, or surface disinfectants used in healthcare as biocides or medical devices).

The exposure assessment strategy between this EU legislation is then compared on the basis of different parameters.

Results and Discussion: The exposure assessment strategies and the depth of the assessment differ between the EU legislation assessed. The extent of the differences as well as the differences in the assessment strategies as such are presented in detail. The reasons for the differences are also evaluated.

Conclusions: With regard to the one substance one assessment strategy, the exposure assessments of different chemicals assessed under different EU legislation could be further harmonised. Several suggestions are made on how such harmonisation could be achieved.

6.07.T-04 Towards a Holistic Approach in Chemical Exposure Assessment: The ExpoAdvance Roadmap

Lara Lamon¹, Alicia Paini¹, James Doyle², Ruth Moeller³, Susana Viegas⁴, Francesco Cubadda⁵, Peter Hoet⁶, An van Nieuwenhuijse³, Henriqueta Louro⁷, Maria Dusinska⁸, Karen S. Galea⁹, Rebecca Canham⁹, Carla Martins⁴, Ana Gama⁴, Vania Teofilo⁴, Maria Joao Silva⁷, Celia Ventura⁷, Paula Alvito⁷, Naouale El Yamani⁸, Manosij Ghosh⁶, Duca Radu³, Marco Siccardi¹, Elise Runden-Pran⁸, Cronan McNamara² and Paul Price¹⁰, (1)ESQlabs, Germany, (2)Creme Global, Ireland, (3)LNS, Luxembourg, (4)ENSP, Portugal, (5)ISS, Italy, (6)KU Leuven, Belgium, (7)INSA, Portugal, (8)NILU, Norway, (9)IOM, United Kingdom, (10)Risk Sciences, Canada

Historically, chemical risk assessments have primarily focused on a chemical-by-chemical approach, evaluating risks associated with exposures from individual sources. However, individuals are exposed to chemicals from multiple sources, as each chemical can exist in various environmental media, foods, and consumer products. This submission focuses on ExpoAdvance, the EFSA roadmap on aggregated exposure, as an example of a holistic approach in the field of chemical exposure assessment. AE is defined as the combined exposure to a chemical from all sources through different routes. Estimating AE is crucial because it is the total dose that determines the overall toxicological effects of a chemical, rather than isolated doses from single sources. AE can be assessed using exposure models or the combination of human biomonitoring (HBM) data and physiologically based kinetic (PBK) models, and needs to be considered in the context of cumulative exposure. The EU Chemical Strategy for Sustainability's proposal for a one substance-one assessment approach underscores the importance of AE assessments across all chemicals as a key component of risk management. EFSA's goal for 2030 is to develop the capability, in collaboration with partners, to efficiently assess AE for chemicals, including contributions from specific sources of exposure in affected populations.

To meet the criteria of building a holistic approach and ensure consistency under different regulatory approaches, the ExpoAdvance roadmap was developed by performing engagement and data collection activities. From this data, we synthesized an initial tiered framework to guide AE assessments that align with EFSA's goals. We identified data gaps by pinpointing where existing AE assessment capabilities fell short of the framework, as well as challenges and barriers to bridging these gaps. These insights informed the development of research Working Areas (WAs) and the subsequent definition of eight project proposals that would allow EFSA and the regulatory ecosystem to embed AE in chemical risk assessment workflows. This contribution highlights two key findings from the roadmap: the proposed framework for AE assessment and the project proposals designed to operationalise it. Emphasis is placed on activities relevant to cumulative exposure and the promotion of holistic approaches in chemical risk assessment.

Disclaimer/Disclosure: The authors acknowledge the European Food Safety Authority (EFSA) and other EU and US stakeholders for their valuable support and guidance in shaping the ExpoAdvance roadmap.

6.07.P-Tu463 Comparing Environmental Risk Assessment Frameworks Under Different EU Legislations

Alessio Ippolito and Franco Ferilli, European Food Safety Authority (EFSA), Italy

This poster investigates the status of regulatory frameworks for the environmental risk assessment of chemicals. The EU represents an interesting case in terms of regulatory system for the environmental risk assessment of chemicals, since many different regulations co-exist.

Most regulations entailing risk assessment of chemicals aim to authorise (or re-authorise) the possibility to produce, sell, and use chemical substances or products containing multiple chemicals along with potential limitations to any of these activities. As such, these regulations are single chemical/product-oriented, and their domain of applicability is dictated by the specific use of the chemical/product. This poster will provide an overview for industrial chemicals, plant protection products, biocides, feed additives, pharmaceuticals for human and for veterinary use. Since the aim of these regulations involves pre-market authorisations, the methodologies used for the risk assessment within these frameworks mainly adopt a prospective approach, where monitoring plays a limited role.

On the contrary, media-specific regulations (such as the Water Framework Directive) rely heavily on a retrospective approach, as their scope is not to grant specific market authorisations but rather to ensure a certain quality standard of the receiving environment.

Quite interestingly, it is not uncommon for the same substance to be assessed under different regulations, depending on its use. For example, several chemicals are used either as PPPs and as biocides, and they are often also regulated as potentially impacting on the water quality.

Several frameworks are analysed in this poster from different perspectives, i.e. in terms of their overarching purpose to the adopted problem formulation (i.e., objects of protection, protection goals, spatiotemporal scales) and the hierarchical structure of the different risk assessment methodologies.

6.07.P-Tu464 Mapping Chemicals Across EU's Legal Frameworks Towards a 'One Substance, One Assessment' Approach

Marlene Ågerstrand, Mathilda Andreassen and Christina Ruden, Stockholm University, Sweden

EU chemicals legislation governs a wide range of chemicals, from those found in everyday items such as healthcare products and food packaging to pesticides, medicines, and waste treatments. Chemicals entering the EU market are typically registered and assessed under applicable laws for hazards and risk management. However, a single substance may have multiple uses and may be assessed under more than one framework, potentially leading to different assessment outcomes. To address these challenges, the European Commission introduced the "one substance, one assessment" approach as part of the Chemical Strategy for Sustainability (CSS). In this study, we mapped substances subject to assessment under multiple legal frameworks to evaluate the scope of the issue and identify challenges to implementation of the one substance, one assessment. Our findings showed that almost one-tenth of the substances identified were listed under more than one framework, highlighting the need for coordination of hazard and exposure assessment across frameworks. One important challenge identified was the notable lack of coherent chemical identifiers available to accurately identify substances across the frameworks, which will complicate efforts to align assessments and re-use and sharing of data. Additionally, we identified the presence of phthalates, bisphenols and PFAS in EU frameworks to illustrate how a group-based approach to chemical assessment could be applied across legal frameworks. As expected, a group-based approach spanned more frameworks compared to the single-substance approach, emphasising the need for coordination and communication across frameworks. Surprisingly, we also found that most of the phthalates, bisphenols and PFAS were not listed in the investigated frameworks while still notified in articles on the EU market according to ECHA's SCIP database.

6.07.P-Tu468 Combined Sewer Overflows - A Neglected Source of Biocide Emissions?!

Christiane Meier and Korinna Ziegler, German Environment Agency (UBA), Dessau-Rosslau, Germany

The sewage system acts as a reservoir for micropollutants, including biocides used as for example disinfectants, insecticides, and preservatives. These enter the sewage system via domestic wastewater or rainwater. During heavy rainfall, treatment plants often reach their capacity and discharge excess wastewater through combined sewer overflows (CSOs) into storage basins or directly into water bodies. This could lead to increased risk for the aquatic ecosystem in urban areas.

In recent years, the German Environment Agency (UBA) has conducted research on biocides in wastewater treatment plant effluents, including CSO discharges. Findings show that even with tertiary treatment, not all biocides are eliminated, allowing their release into water bodies. CSOs, lacking purification steps, discharge biocides directly.

Above all, substances used as material preservatives such as terbutryn, diuron, isoproturon, and isothiazolinones were detected in effluents. Imidacloprid and permethrin, found additionally in CSO samples, are particularly a cause of concern due to their high toxicity to aquatic life at low concentrations.

Climate change projections indicate more frequent, intense precipitation, increasing the relevance of CSO pathways in urban areas. Current environmental risk assessments for biocides exclude CSO inputs, considered negligible due to assumed rare occurrence. However, UBA findings and CSO occurrence data show that this pathway should be included in risk assessment of some biocides to better protect aquatic ecosystems.

6.07.P Environmental Risk Assessment Under Biocides and Other EU Legislations

6.07.P-Tu463 Comparing Environmental Risk Assessment Frameworks Under Different EU Legislations

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6.07.P-Tu465 Advancing the Environmental Risk Assessment of Pesticides to Better Protect Biodiversity Under PARC

Johan Axelman¹, Annette Aldrich², Yann Devos³, Sabine Duquesne⁴, Vanessa Mazerolles⁵, Natasha Molgaard⁶, James Henty Williams⁶, Gustaf Bostrom⁷, Matthias Liess⁸, Ralf Bernhard Schaefer⁹ and Jose V. Tarazona¹⁰, (1)Swedish Chemicals Agency (KemI), Sweden, (2)Federal Office for the Environment (FOEN), Switzerland, (3)European Food Safety Authority (EFSA), Italy, (4)German Environment Agency (UBA), Germany, (5)French Agency for Food, Environmental and Occupational Health and Safety (ANSES), France, (6)Aarhus University, Denmark, (7)Swedish University of Agricultural Sciences (SLU), Sweden, (8)Helmholtz Center for Environmental Research (UFZ), Germany, (9)Research Center One Health Ruhr, University Duisburg-Essen, Germany, (10)Instituto de Salud Carlos III (ISCIII), Spain Within the EU Partnership for the Assessment of Risks from Chemicals (PARC), the Activity 6.4.4 Risk assessment to support and promote efficient overall protection of biodiversity explores new lines of research to advance the environmental risk assessment (ERA) of chemicals within and beyond their current regulatory ERA frameworks (with an initial focus on chemical pesticides). The ambition is to foster the development and implementation of systems-based approaches for ERA that address regulatory needs.

The interconnected lines of research aim at: (1) the clarification of regulatory needs to ensure regulatory relevance and uptake of outcomes and bridge the research policy gap; (2) the updating and modernising of ERA approaches to ensure fitness for purpose; (3) the benchmarking of ERA against reality to simplify and speed up the ERA process and increase predictability; (4) the increase of ecological realism of ERA to deliver more realistic, context-dependent ERA predictions and risk mitigation/sustainable use measures and (5) the application of a systems-based approach for better connecting stakeholders and integrating data, knowledge and expertise within and across regulatory frameworks.

PARC 6.4.4 plays a unique role to advance the ERA framework for PPPs towards a systems-based approach in the EU, as it explores scientific avenues (including case studies) to simplify and speed up ERA methodologies, increase ERA's ecological realism, improve ERA's agility and ease the transition towards a systems-based approach to PPP ERA.

Issues with the PPP ERA identified in previous regulatory research projects (i.e., PERA, ZAPID) were acknowledged and confirmed by risk assessors engaged in PARC 6.4.4 through an online survey and workshops. We will present preliminary results of insights and feedback gain from risk assessors as part of the survey, and subsequent workshop exchanges. In addition, synergies with other EU projects that aim to advance the ERA of chemicals will be presented, and means to strengthen the collaboration among these projects, as well as stakeholders within and across regulatory frameworks will be explored.

By connecting relevant projects and stakeholders within and outside PARC, PARC 6.4.4 seeks to engage towards the establishment and implementation of a partnership on Next-Generation ERA (NG ERA) of chemicals.

6.07.P-Tu466 What Will be ECHA's Role in Protecting Environmental Waters in the EU?

Pia Talja and Chrystelee Tissier, European Chemicals Agency (ECHA), Finland

The European Chemicals Agency (ECHA) is set to embark on a new mandate to work on the water protection directives, including the Water Framework Directive (WFD), Environmental Quality Standards Directive (EQSD), and Groundwater Directive (GWD), as proposed by the European Commission in the revision of those directives under discussion. ECHA will be supporting the Commission in, for example, identifying priority substances and proposing environmental quality standards (EQS), establishing groundwater pollutant lists and quality thresholds, and identifying substances for watchlists for both surface water and groundwater.

In preparation for these new responsibilities, ECHA has started to familiarise itself with the future tasks by

understanding better the scope of these tasks and how they have been managed historically. This includes analysing new requirements from the directives, such as the mandatory groundwater watchlist, identifying key stakeholders, and observing the work of the Commission's working groups. Regular exchanges with the Commission, the European Environment Agency (EEA), and the Joint Research Centre (JRC) have been crucial.

ECHA faces several key challenges in this transition. The long history of water protection work being handed over requires the acquisition of new expertise and the establishment of a suitable working relationship with existing Commission working groups. Defining ECHA's role and potentially introducing some changes to past practices will be needed. Additionally, both the Risk Assessment Committee (RAC) and the Socio-Economic Analysis Committee (SEAC) will be involved in this work and ECHA needs to figure out how to best use the committees and their expertise.

However, there are significant opportunities as well. ECHA's in-house expertise is extensive, and there is potential to create better synergies between water protection efforts and existing tasks. For instance, the water protection work is expected to provide additional information that could inform further regulatory actions, such as substance restriction. Streamlining tasks for surface and groundwater pollutants where relevant and integrating various chemical-related legislations under one roof, will help ensure a more holistic approach. This aligns with the broader goal of the One Substance, One Assessment legislation, promoting a comprehensive and unified regulatory framework for managing chemical pollution.

6.07.P-Tu468 Combined Sewer Overflows - A Neglected Source of Biocide Emissions?!

Christiane Meier and Korinna Ziegler, German Environment Agency (UBA), Germany

The sewage system acts as a reservoir for micropollutants, including biocides used as for example disinfectants, insecticides, and preservatives. These enter the sewage system via domestic wastewater or rainwater. During heavy rainfall, treatment plants often reach their capacity and discharge excess wastewater through combined sewer overflows (CSOs) into storage basins or directly into water bodies. This could lead to increased risk for the aquatic ecosystem in urban areas.

In recent years, the German Environment Agency (UBA) has conducted research on biocides in wastewater treatment plant effluents, including CSO discharges. Findings show that even with tertiary treatment, not all biocides are eliminated, allowing their release into water bodies. CSOs, lacking purification steps, discharge biocides directly.

Above all, substances used as material preservatives such as terbutryn, diuron, isoproturon, and isothiazolinones were detected in effluents. Imidacloprid and permethrin, found additionally in CSO samples, are particularly a cause of concern due to their high toxicity to aquatic life at low concentrations.

Climate change projections indicate more frequent, intense precipitation, increasing the relevance of CSO pathways in urban areas. Current environmental risk assessments for biocides exclude CSO inputs, considered negligible due to assumed rare occurrence. However, UBA findings and CSO occurrence data show that this pathway should be included in risk assessment of some biocides to better protect aquatic ecosystems.

6.07.P-Tu469 The Environmental Impact of AAV Gene Therapy vectors

Tobias Fleischmann, Pfizer, Germany

Background: Adeno-associated viruses (AAVs) have become the predominant vectors for in vivo gene therapy (GT). Despite their widespread use, the environmental risks associated with recombinant AAV (rAAV), particularly during the shedding phase, remain underexplored. This pair of studies aims 1) to address the biocontainment and hygiene requirements for handling secreta/excreta of GT patients and 2) to assess the environmental fate of shed rAAV particles.

Methods: Feces and urine samples from non-human primates treated with an AAV9-based GT vector (2E13 vector genomes per kilogram body weight) were analyzed for vector presence and cell-binding capacity. Additionally, the stability of four rAAV vectors (based on wildtype AAV serotypes 2, 3, 6, 9) was evaluated in activated sludge to simulate wastewater treatment facility (WWTF) conditions.

Results: Both feces and urine samples contained vector particles capable of binding to cells in a cell culture system. However, these shed particles exhibited a 2-3 orders of magnitude lower affinity to cells compared to novel control rAAV vector. In the environmental fate study, detectable virions in activated

sludge decreased rapidly within hours, reaching the lower limit of quantitation by day 7. Under abiotic conditions, the half-life of rAAV virions was approximately 7 days.

Conclusions: The lower binding capacity of shed vector particles suggests that current stringent biocontainment and hygiene measures for GT patients could be relaxed. The rapid degradation of rAAV particles in WWTFs indicates that they do not pose an environmental threat. These findings support a more relaxed, risk-based regulatory approach for medicinal GMOs, potentially simplifying the regulatory review process and encouraging a science- instead of fear-based approach to the safety protocols for gene therapy applications, enabling faster access to GT to more patients.

Disclaimer/Disclosure: Both studies were funded by Pfizer Inc.

6.07.P-Tu470 Aerial Application of Plant Protection Products with Drones in Germany

Bianca Kuhne and **Jonas Schartner**, *Federal Office of Consumer Protection and Food Safety, Unit Environment, Germany*

In 2021 aerial applications of plant protection products with drones were approved in Germany for the first time.

According to the sustainable use directive 2009/128/EC aerial spraying is prohibited. A derogation is only possible in special cases under certain conditions. According to the German Plant Protection Act two exemptions are foreseen:

Aerial applications (helicopter, drones) are only allowed in steep slope vineyards and forest canopies. Drift values have been collected for drones only in steep slope vineyards at the moment. For drones, so far only applications of fungicides in steep slope vineyards were granted.

The approval or authorisation of aerial uses (including drones) of plant protection products are outlined in § 18 of the German Plant Protection Act.

The assessment is carried out by the competent authorities regarding efficacy, health and environment. For all uses of plant protection products with drones risk mitigation measures apply. For example, uses must be complied with a certain distance above the crop and flying speed.

The application of a plant protection product using aircraft requires an additional authorisation of the competent authorities of the German Federal States.

There might be a potential for drone application in other crops. It would allow an application in difficult terrain or a treatment of only certain areas in the field. The challenges are the current legal framework conditions within the EU and DE and the drift and impact in comparison with conventional spraying in field crops.

The poster will give an overview of the authorisation process for applications of plant protection products with drones in Germany. It will show the current status for risk assessment and management. Furthermore, it will highlight the potential and challenges for a future possibility of applications with drones in further crops.

6.07.P-Tu471 Beyond Default Mixture Allocation Factor-settings: How Can We Increase the Applicability and Scientific-relevance of Mixture Risk Assessments for Metal(oid)s?

Charlotte Nys¹, **Karel Viaene¹**, **Karel Vlaeminck¹**, **Maria Laura De Donno²**, **Marius Schmitt²**, **Koen Oorts¹**, **Marnix Vangheluwe¹**, **Hugo Waeterschoot³**, **Lara Van De Merckx³** and **Karel De Schampheleere²**,
(1)ARCHE Consulting, Belgium, (2) Ghent University, Belgium, (3)Eurometaux, Belgium

The European Commission calls in the Chemicals Strategy for Sustainability for a systematic investigation of the impact of combined exposure into chemical risk assessments under REACH. To cover for such effects a default Mixture Allocation Factor (MAF) of 5 is under discussion, which most probably will imply that the safety limit of single substances will be reduced. Analysis shows that the implementation of a default MAF will have a large impact on the environmental risk assessment of inorganics (including metals and metalloids). Given that metal(oid)s are naturally occurring, the implementation of such a MAF may result in exceedances of safety limits at natural background concentrations, suggesting a combined risk. However, to our knowledge, a specific MAF for metal(oid)s has not yet been derived, as most existing studies do not consider metal(oid)s. In addition, default MAF calculations include different levels of conservatism which originate from the underlying mixture model, i.e., concentration addition (CA). These conservatisms include that I) all substances have the same mode of action, II) all substances contribute to the mixture toxicity, and III) for all substances the same species drives the environmental threshold (e.g., PNEC).

To anticipate the mixture toxicity correction under REACH, we evaluated the relevance of the proposed default MAF for metal(oid)s. This was done by calculating a metal(oid)-specific MAF (with the MAFceiling-approach) using European-wide monitoring databases, considering both the presence of

metal(oids) and their co-occurrence with organic substances in freshwater systems. In addition, the conservatism in default MAF settings was assessed via the I) Mixture Interaction Factor (MIF), which expresses the conservatism of CA-mixture predictions relative to observed mixture effects at regulatory relevant effect levels ; and the II) Margin of Safety (MoS) expressing the conservatism of applying CA-based risk assessment models at the ecosystem level (PNEC) rather than at the species-level (EC10). In addition, based on these calculations a tiered scheme to incorporate mixture risks in environmental compliance assessment of REACH-regulatory dossiers for metal(oid)s is proposed. The proposed scheme incorporates default MAF settings as a baseline screening tier, and scientific-relevant refinements (e.g., MIF, MoS, bioavailability, biodiversity-effects) in higher tiers.

Disclaimer/Disclosure: This work was funded by Eurometaux.

6.07.P-Tu472 Ecotoxicological Biomonitoring Using In Situ Caging of Gammarus sp. (crustacea): Large and Local Scale Implementation in France

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The WFD requires European Union member states to protect and restore water bodies to reach good chemical status. To this end, France has implemented, a strategy based on active biomonitoring using in situ caging of Gammarus sp. to measure priority substances, check the compliance with biota-EQS (i.e., environmental quality standard) and assess trends with national thresholds, called BBAC (i.e., bioaccumulation background assessment concentration). In parallel with this chemical monitoring, this method offers the opportunity to carry on ecotoxicological measurements. Indeed, assays based on biological responses (i.e., bioassays) are relevant to evaluate both exposure and effect of chemicals as they integrate all bioavailable chemical substances. Deployment has been performed on 5 hydrographic basins based on 3 campaigns per year from 2019 to 2024. Unspecific (survival, feeding activity and fertility rate) and specific (acetylcholinesterase activity and endocrine disruption) biological markers were measured. This proposal aims to (1) present feedback on the collected data over 6 years and (2) present results for two case studies at large and local scales.

Gammarids are collected in breeding ponds, kept under laboratory conditions and calibrated before in situ exposure. Males and females are distributed respectively in 5 cages (20 males per cage) or 4 cages (7 females per cage). Depending on bioassays, in situ exposure lasted for one (feeding and AChE activity) to three (fertility rate and ED effect) weeks. Endpoint measurement was performed according to French AFNOR standards protocols XP-T90-722-1, XP-T90-722-2 and XP-T90-722-3.

Between 2019 and 2024, 3,079 caging were performed (i.e., 358 stations and 16 campaigns). 97% of the caging systems were successfully retrieved.

The present study shows that in situ caging of Gammarus sp. has been successfully implemented in France since 2019 to assess water bodies ecotoxicity. Concerning the large-scale study, i.e., French water agency basins, the results highlight that the feeding and fertility rates were the most discriminating responses. This allows the ranking of stations according to their level of toxicity and monitoring trends over time. In the same way, at a local scale (i.e., river) in situ caging of Gammarus sp. has proven to be an effective tool to objectify sources of toxicity.

6.07.P-Tu473 Current Risk Assessment of Non-Target Terrestrial Plants Under the Regulation (EU) 1107/2009: Deficit Analysis and Recommendations for the Revision of the Terrestrial Guidance Document

Harry Byers¹, Magali Sole¹, Caroline Baudson², Laura Mathieu², Hana Kubatova-Hirsova³, Sweba Ghani¹, Stephan Brendel¹, Cecile Perillon¹ and Steffen Matezki¹, (1)German Environment Agency (UBA), Germany, (2)Health Food Chain Safety Environment, Belgium, (3)Central Institute For Supervising and Testing in Agriculture, Czech Republic

The current Terrestrial Guidance Document (SANCO/10329/2002) was adopted 22 years ago, and 7 years

before the Regulation (EC) No. 1107/2009. It neither reflects the latest scientific developments nor does it adequately incorporate the provisions of the Regulation (EC) No. 1107/2009. The European Food Safety Agency (EFSA) has acknowledged various shortcomings in its review of the current state of scientific knowledge for the risk assessment of Non-Target Terrestrial Plants (NTTP) exposed to pesticides (EFSA 2014 Scientific Opinion). Since then, no progress has been made in the development of a terrestrial guidance document. Finally, in June 2024, EFSA received a mandate from the European Commission for the revision of the Terrestrial Guidance Document. Due to the long delay there is the question whether EFSA's reviews from 2014 remain up to date.

This study focuses on the NTTPs and aims to identify recent scientific advancements since the publication of the EFSA 2014 Scientific Opinion. To achieve this, we carried a comprehensive literature review. In total, 2137 papers were identified following a query on various databases (Scopus, web of science), combined with a snowball search. After an initial assessment, 192 studies were considered relevant for the revision of the terrestrial guidance document for NTTPs and attributed to different topics of the NTTP risk assessment (e.g. Effect, Exposure, Calibration, Higher-tier).

Besides identifying new deficits, the definition of the deficits presented in EFSA 2014 has been also updated. Key findings included: more recommendations on the representativeness of the species tested, the mode of action of the substance under evaluation and improvements to exposure models (e.g. implementation of a MAF). More guidance for tests evaluation, SSD simulation or higher tier studies are needed. One of the main deficit of the risk assessment scheme was the lack of calibration for the tiered approach. As already reported in EFSA 2014, the need to develop specific in-field Protection Goals for NTTPs, to address their role in habitat and food web support, was confirmed.

This aligns with the general protection goals outlined in (EC) 1107/2009 and related legislative documents. Considering these recommendations in the forthcoming revision of the Terrestrial Guidance document would make a significant regulatory advancement regarding a better protection of NTTPs and a further strengthening of biodiversity as legal protection goal.

6.07.P-Tu474 Reproductive Endpoints and Phenological Stage Exposed in Non-Target Terrestrial Plants Studies: A Meta-Analysis Using a Percentile Approach and Consequence for Risk Assessment Under (EU) 1107/2009

Sweba Ghani¹, Cecile Perillon¹, Thomas Graff¹, Harry Byers², Caroline Baudson³, Hana Kubatova-Hirsova⁴, Stephan Brendel¹ and Magali Sole¹, (1)German Environment Agency (UBA), Germany, (2)German Environment Agency (UBA), France, (3)Health Food Chain Safety Environment, Belgium, (4)Central Institute For Supervising and Testing in Agriculture, Czech Republic

Currently, only vegetative endpoints are evaluated and considered in the risk assessment of Non-Target Terrestrial Plants (NTTPs) under the European Regulation (EU) 1107/2009 for pesticides. However, evaluating pesticide effects across the entire plant life cycle from seed germination and seedling stages to juvenile development, flowering, seed production, and subsequent germination is essential for assessing their overall impact on natural plant populations and safeguarding biodiversity (EFSA 2014).

While prior studies have shown that vegetative endpoints may not be adequately protective with respect to reproductive endpoints in NTTPs, a recent meta-analysis from 2019 identified no NTTP taxa as particularly sensitive to reproductive outputs. However, this analysis relied on comparing average values. This statistical approach is nonetheless considered insufficient for regulatory purposes as it might not account for sensitive species. Consequently, for regulatory purposes, percentile-based approaches are usually applied to ensure protection of a broader range of species, such as the 10th percentile, rather than relying solely on central measures like the mean or median.

This study aims to update the dataset used in the meta-analysis from 2019 with data from the past decade and reanalyze it using a percentile-based approach. In addition to comparing vegetative and reproductive endpoints, we examine the impact of the phenological stage during exposure. Specific recommendations will be made regarding the inclusion of reproductive endpoints in ecotoxicological testing and/or in risk assessment. The findings are intended to support the forthcoming revision of the Terrestrial Guidance Document launched by the European Commission in June 2024.

6.07.P-Tu475 German Court Judgement: The Right to Environmental Information Grants Access to ERA Data and Studies from the Human Medicinal Product Authorisation Procedure – Recommendations for its Implementation

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The pollution of pharmaceuticals in the environment is of concern and risk mitigation measures should further be developed. Environmental risk assessment (ERA) is part of the EU authorisation procedure of veterinary /human medicinal products (VMP/HMP) since 1998/2006, but the availability of these data for the field of environmental protection is limited.

In a German court judgement of historic importance, the relationship between access to environmental information here data and studies of the ERA of medicinal products for the plaintiff on the one hand, trade and business secrets or intellectual property rights from the pharma industry on the other hand was fundamentally clarified:

The ERA data and studies of active pharmaceutical ingredients of the authorisation procedure are environmental information on emissions. Thus, trade or business secrets cannot be held against the right to environmental information. Intellectual property rights was from the pharma industry not convincingly presented, because the procedures required by the EMA guidelines using standardized methods, even if it were, there would be a weighed up in which the public interest would prevail.

In conclusion, the competent authority is required to grant the plaintiff access to the complete ERAs, i.e., all documents of the submitted dossier required under Annex 1 part I. module 1. point 1.6. "environmental risk assessment" of Directive 2001/83/EC and information for each of the selected substances (medicinal products) (ECLI:DE:VGK:2023:0713.13K5068.18.00).

By clarifying the accessibility of ERA data and ERA studies, the German court has made a groundbreaking decision with European implications [1].

Beside the judgement, recommendations for its implementation, derived on a multidisciplinary stakeholder workshop with participants from e.g. Federal Environmental Agency, waste water treatment and drinking water authorities, universities and research institutes will be presented.

6.08 Regulatory Risk Management of Chemicals Integrating Risks, Impacts and Socio-Economic Assessments for Robust Policy Decisions

6.08.T-01 Why Should we Integrate Risks, Impacts and Socio-economic Assessments for Speeding-up the Phase-out and Substitution of Harmful Chemicals?

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The European Chemicals Strategy for Sustainability Towards a Toxic-Free Environment intends to facilitate decision-making and regulatory efficiency towards a fast phase-out of the most harmful chemicals by applying the essential use (EUSE) concept. In REACH authorisation and restriction procedures, in contrast, socio-economic analysis (SEA) has served as a decision-support tool for regulatory risk management of chemicals. It has repeatedly been argued that the SEA approach is too inert to ensure a quick phase-out or substitution of chemicals. In contrast to REACH, a comparative assessment as required under the Biocidal Products regulation (BPR) has not been worked out in detail yet. Based on an explorative analysis, we spell-out key requirements for the EUSE concept to achieve its aims, and discuss what this implies for impact assessment and cross-disciplinary integration of information. Our analysis reveals that, compared to a SEA and other forms of comparative approaches used in European chemicals legislations, decision-making based on the EUSE concept considers the benefits of regulatory measures only. Information about negative impacts or damage costs of a ban (in case the use of a chemical deems non-essential), or an exemption of a ban (in case the use of a chemical deems essential), is ignored. Such simplification will likely reduce the need for cross-disciplinary integration of knowledge and data. While this may save time, disregarding such relevant information can lead to robust policies only if specific requirements are met. It requires, for instance, to reliably detect chemical uses which are truly (non-)essential, i.e., for which it is clear a priori that all positive impacts (social benefits) of a derogation, a phase-out or a substitution exceed all negative impacts (social costs). This process requires to define unambiguous criteria for assessing the necessity and criticality of chemical uses in relation to societal goals, including measurable indicators, assessment tools and, ultimately, decision-rules. Likewise, criteria and corresponding methods for the assessment of acceptable alternative substances and technologies have to be defined. For the majority of harmful chemicals, unequivocal conclusions on the (non-)essentiality of a chemicals use will likely be impossible. This provides strong arguments for strengthening the integration of risk and impact information into SEAs, capturing all non-market impacts of chemical policies.

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6.08.T-02 An Ecosystem Service Impact Assessment Framework Applicable to Any Chemical Pollutant

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"Chemical pollution affects species, ecosystems, and their ability to deliver services, creating costs that must be balanced against economic benefits. Managing these impacts is challenging due to the diversity of pollutants to be assessed, extensive data gaps, and insufficient knowledge on the links between ecotoxicology and ecosystem services. This issue often lead to a reliance on precautionary risk assessments. To address the challenge of chemical impact assessment in a more holistic manner, we outline a proposed framework that integrates information on chemical hazard, exposure, ecological effects, and impacts on ecosystem services. Steps of the framework are detailed below.

- Step 1: Hazard Ranking – Chemicals are ranked into potency groups using HC50 values from species sensitivity distributions (SSDs) and alternative data (e.g. from QSARs). Additional hazards elevate potency levels.
- Step 2: Exposure Ranking – Chemicals are ranked by environmental concentration and detection frequency, adjusted for persistence and mobility.
- Step 3: Community Impact Assessment – Combines potency and exposure to estimate species-level impacts and taxonomic group sensitivity.
- Step 4: Ecosystem Service Impacts – Links taxa declines to ecosystem processes and services, quantifying reductions in services like pollination and nutrient cycling.

The framework was tested for three case study chemicals. For fipronil, the high potency and exposure could be linked to impacts on insects and nematodes. For copper, moderate-high potency and exposure could be linked to affects on plants, microbes, and some invertebrate taxa. For perfluorohexane sulfonic acid the framework indicated effects on vertebrates, affecting services like game and food provision.

The proposed assessment framework links chemical hazards and exposure to ecosystem impacts, enabling assessments for chemicals with varying data levels. Demonstrated through case studies, the framework offers insights into the ecosystem service costs of chemical pollution, guiding risk management and decision-making.

6.08.T-03 Integrating Risk and Socio-Economic Assessments to Support Decision-Making in Soil Remediation

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In an era of pressing environmental challenges, there is a growing need for comprehensive decision-making frameworks that integrate scientific, economic, and social dimensions. Traditional approaches to chemical risk management, relying predominantly on risk assessments, fail to adequately address the socio-economic implications of ecological hazards. To bridge this gap, we propose an integrative framework combining ecotoxicological risk assessment with socio-economic evaluation, aimed at enhancing the depth and relevance of decision-making processes for soil remediation. As part of the EU-ARAGORN project, we conducted a systematic literature review to examine how existing socio-economic analyses (SEA) have addressed the impacts of soil remediation strategies. The review highlights the lack of a harmonized approach to SEA, emphasizing that the selection of remediation impacts and the parameters for assessing impacts is case-specific. The expected impacts of remediation will differ, for example depending on pollutant properties, spatial scale, remediation technologies, and intended land-use after remediation. Building on lessons from existing guidance frameworks and advancements in toxicology and economic theory, we developed a flexible decision-support tool tailored to varying data availability and site characteristics for different methodological levels (T1-T3). The framework evaluates social costs and benefits including environmental, human health, and (wider) economic impacts. This allows for qualitative, quantitative, or monetized comparisons of remediation strategies. Moreover, these levels ensure adaptability to diverse contexts, supporting multi-criteria decision-making and stakeholder engagement. With an application to a case study in Moldova Noua (Romania), the framework aims at supporting decision-making processes for soil remediation not only by facilitating the translation of scientific data into accessible formats such as spatial maps, but also informed choices by integrating

ecological and socio-economic considerations. The framework thus shows the importance of sustainable and economically viable soil remediation strategies.

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6.08.T-04 Using Price Incentives to Substitute Hazardous Chemicals in the European Union

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The presence of hazardous chemicals poses significant threats to human health and ecosystems. Substances of Very High Concern (SVHCs), as identified under the EU's REACH Regulation, are among the most problematic, yet progress in reducing their use remains limited due to regulatory challenges and high administrative costs. This study proposes the introduction of economic incentives, specifically fees, to accelerate the substitution of SVHCs with safer alternatives.

We evaluate two fee models tailored to the EU context: (1) company-based annual fees, inspired by Massachusetts Toxics Use Reduction Act (TURA), and (2) tonnage-based fees, comparable to the EU's plastic tax. Using data from the European Chemicals Agency (ECHA) on SVHC usage and authorization applications, the study models the potential substitution effects and revenue generation under various scenarios.

Results indicate that a tonnage-based fee of 800 per tonne could reduce SVHC usage by up to 79% in high-volume sectors, while generating significant revenues for reinvestment in research and innovation. Conversely, company-based fees, though less effective in driving large-scale substitution, enhance compliance and provide targeted incentives for smaller firms to explore safer alternatives. Annual revenues from this approach could range between 2 million and 27 million, depending on the scope and design.

Our findings demonstrate that economic instruments like SVHC fees can complement existing regulations by promoting cost-effective substitution, innovation, and reduced health and environmental risks. Further research is needed to refine fee structures and explore mechanisms for revenue recycling to support industry transitions toward non-toxic solutions.

6.08.P Regulatory Risk Management of Chemicals Integrating Risks, Impacts and Socio-Economic Assessments for Robust Policy Decisions

6.08.P-Tu477 A Framework to Break Down Silos Across Disciplines and Enable More Holistic Decisions and Communication: Case Study for Octocrylene Environmental and Public Health

Jennifer K. Saxe, Kurt A Reynertson and Sagar Thakali, Kenvue, United States

Octocrylene, an ultraviolet light filter (UVF) ingredient in sunscreens, has been proposed for restriction in the EU under REACH, underpinned by a regulatory environmental risk assessment (ERA). In the US, an expert committee convened by the National Academies of Sciences, Engineering, and Medicine published a comprehensive review of the environmental fate, exposure, and effects of UVFs. They identified key knowledge gaps preventing rigorous ERAs for UVFs and raised concerns that regulatory decisions about sunscreen ingredients could influence human behavior and health outcomes. Specifically, communication regarding the environmental safety of UVFs could result in negative perception and reduced use of sunscreen products with established cancer prevention benefits.

We created a framework for quantitatively linking decision-making and communications about sunscreens to changes in their public perception, availability, use and accompanying health outcomes. This framework applies to any scenario where communication about one set of product attributes (e.g., desirability, safety) could result in unintended harm through a cause-and-effect chain leading to adverse public health outcomes. The framework uses data, models, and collaboration across disciplines, including environmental, behavioral, and medical sciences, economics, public health, and epidemiology, and is meant to break through specialists silos, leading to better decisions and communications.

The framework is applied to the EU octocrylene review, which occurs in a region where numerous alternative UVFs are available due to the classification of sun protection products as cosmetics. If a decision is made using a conservative, precautionary approach for environmental safety, any reduced sunscreen use and concomitant adverse health outcomes could be mitigated by ready access to alternative UVFs in the EU. However, in the US there is a relative lack of alternative UVFs because sunscreens are

regulated as medicinal products, creating barriers to market entry. We show that, due to rapid global information dissemination, public communications about any EU octocrylene decision could have unintended negative consequences for US public health.

This framework provides a more holistic view, linking ERA, perception and behavior, and public health, and demonstrates the importance of collaboration across disciplines and geographies to improve communications and avoid unintended public health outcomes.

6.08.P-Tu478 Transferable Quotas for PFAS in the EU

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Four EU Member States and Norway made a proposal to restrict Per- and Polyfluoroalkyl Substances (PFAS) on 13 January 2023. During public consultation on the restriction ECHA received an unprecedented number of 5600 comments. These alone already demonstrate the ubiquitous use of PFAS in the society. It has been very challenging for ECHA's scientific committees to give opinions on the proposed restriction and ECHA has not been able to tell when its opinion would be adopted. This indicates that the Commission will also have substantial challenges to decide on how PFAS should be restricted in an proportionate manner.

In the paper we discuss if it was possible to set a condition based on transferable quotas allowing the continued manufacture, placing on the market or use of PFAS instead of a ban if it would lead to disproportionate socio-economic impacts. Alternatively, a specific legal instrument could be created in the EU to complement the restrictions on PFAS.

The use of transferable quotas would be a new way of encouraging substitution by setting an ambitious reduction target for all PFASs and letting the market operators, i.e. the manufacturers and importers of the PFAS, to find cost-effective ways of reaching the target through transferable quotas. Such system is already being used under EU's Fluorinated greenhouse gas regulation (2024/573), which phases out by 2050 industrial chemicals -- which are also PFASes. This regulation includes all necessary elements and would thus be a relevant starting point also for PFASes.

EU's Emission Trading System in greenhouse gases has some helpful elements that could be included in the transferable quota system for PFASes, in particular the possibility to bank and transfer quota. With some modifications, in particular through a possibility of banking and transferring the quota more flexibly, and adapting the infrastructure -- e.g. the F-gas portal to an PFAS-portal -- the proposed system would be encourage innovation and thus substitution and be at the same time market friendly.

We will also discuss briefly which PFAS could be regulated through the transferable quota system.

The decision on a transferable quota system would be made in the EU based on qualified majority, like a revision of the REACH Regulation. It could be done as part of the revision of the regulation, either concurrently or in parallel.

6.08.P-Tu479 Chemical Stock Pollution Modelling to Determine the Environmental Impact of Emission Intervention Measures for Persistent Mobile and Toxic Substances: The case of PFOA

Johannes Meesters, Joris T.K. Quik, Emiel Rorije, Matthias Hof, Silke Gabbert and Arianne de Blaeij, RIVM, Netherlands

Persistent, mobile and toxic substances pose increasing long-term environmental and human health risks, because environmental concentrations incline as long as emission rates exceed degradation rates. Intervention measures, for instance for perfluoroalkyl and polyfluoroalkyl substances, minimize emissions, but the pollution stock due to past emissions can remain high for decades or even centuries. We present an approach to determine the environmental stock arising from different regulatory interventions affecting emissions from production, use, and end-of-life of products applying dynamic stock pollution modeling with the multimedia fate model SimpleBox. Regulatory interventions differ with regard to the type and timing of emission reduction scenarios. The assessment is conducted for perfluorooctanoic acid (PFOA) in Western Europe and its environmental stock in air, water, sediments and soil predicted over the period of 1951-2050. The interventions evaluated are (i) the industrial initiatives in 2002 to phase-out PFOA production, its use as formulation agent and on-site emissions and (ii) the European Union's legal restrictions on PFOA that came into force in 2020. Life cycle mass flow data was used to construct a realistic emission scenario including interventions, which is compared to five hypothetical scenarios in

which (i) intervention measures were never taken, (ii) no new PFOA was produced or used after 2002 (iii) restrictions in 2020 never came into force (iv) reductions of the amount of PFOA emitted per amount used or produced at industrial plants were the only interventions and (v) the production of PFOA and its use was reduced with 50% after 2002. Results demonstrate that environmental stocks in air, soil and sediments on a continental scale could effectively be reduced after emission interventions taken in 2003 and 2020. However, PFOA emitted in the past is hardly being degraded, but was instead displaced from continental fresh waters to global ocean waters. The stock in global oceans as a result of past Western European emissions poorly declines but would continue to incline if no interventions were taken. These insights demonstrate that dynamic stock modeling proves to be a useful tool for evaluating the effectiveness of regulatory interventions usually affecting emissions. This gives relevant and clear decision-support to prioritizing and selecting risk management measures to policy makers, which can be integrated in socio-economic analyses.

6.08.P-Tu480 A Stepwise Prioritization Approach Towards Effective Regulatory Measures of PMT/vPvM Substances in the REACH Registration Database

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PMT (Persistent, Mobile, Toxic) and vPvM (Very Persistent, Very Mobile) substances pose significant intrinsic hazards due to their ability to cause long-lasting and diffuse contamination of water resources, which can lead to adverse effects on human health and the environment. The Classification, Labelling and Packaging (CLP) Regulation ((EC) No 1272/2008) ensures a high level of protection of health and the environment. A core principle is the 'self-classification' of a substance or mixture by the manufacturer, importer or downstream user. The classification is based on the hazardous properties of a substance or mixture and not on the likelihood of exposure and risk considerations. On 20th April 2023 the new hazard classes PMT and vPvM entered into force.

Prioritization is crucial for regulation as it enables the efficient allocation of resources to address the most significant risks posed by hazardous substances, ensuring that regulatory actions are targeted where they are needed most. It helps identify substances that require immediate attention, facilitating timely risk management measures to protect public health and the environment. Additionally, prioritization highlights data gaps and areas needing further research, guiding stakeholders in developing effective strategies for monitoring and remediation efforts.

The stepwise prioritization approach towards effective regulatory measures of PMT/vPvM substances in the REACH registration database developed by the German Environment Agency (UBA) consists of three steps. The first step involves the identification and classification of the intrinsic hazard of PMT/vPvM substances, focusing on their potential to cause harm. The second step focuses on the prioritization of PMT/vPvM substances for regulation based on their and exposure levels and potential risk to drinking water sources.

The third step involves the prioritization of regulatory measures based on their effectiveness. This step ensures that regulatory actions are targeted, effective, and proportionate to the risks posed by PMT/vPvM substances. Ultimately, this step aims to implement regulatory actions that effectively minimize the environmental emissions of hazardous PMT/vPvM substances while promoting safer alternatives.

The stepwise prioritization approach is a critical tool for identifying effective regulatory pathways to mitigate emissions of high-priority PMT/vPvM substances, such as 1H-Benzotriazole, 1,4-Dioxane, and Melamine.

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6.08.P-Tu481 An Attempt to Establish Recycled Plastic Grades Focusing on Plastic Additives to Enable Appropriate Recycling

Kyoko Ono, Masashi Gamo, Naohide Shinohara, Naoya Kojima and Isamu Ogura, Advanced Industrial Science and Technology, Japan

Promoting plastic recycling is an increasingly important issue from the perspective of resource recycling

and preventing plastic pollution. Determining the hazards and risk of the chemicals in the recycled plastic is difficult because the recycled plastics are made from a variety of plastic wastes, such as used plastics and leftovers from the production of plastic products. However, if such risks are not assessed and managed, concerns remain that resource-efficient recycling of plastics come to a standstill or remain limited.

This study aims to develop a grading concept for waste plastics and recycled plastics. The risks associated with the use of recycled plastics come from plastic additives, contaminants, and by-products contained in plastics (hereinafter referred to as plastic chemicals). Therefore, a risk-based grading approach for recycled plastics is developed by conducting a trial grading of recycled plastics based on a risk assessment framework for chemicals contained in plastics.

We assumed that the grade of recycled plastic was determined according to the risk of the chemical substances contained in the plastic. This risk corresponds to the hazard ratio related to the risk of plastic chemicals for a specific recycled plastic in a specific use case. The hazard ratio is expressed as follows: $C \times L \times M \times E / T$

where C is the concentration of the plastic chemical(s) in the recycled plastic; L is the leaching characteristic of the plastic chemical(s); M is the migration factor for correction according to the scenario/exposure route; E is the exposure factor of the usage scenario; and T is the toxicity of the plastic chemicals, for example, the upper exposure limit. If it can reasonably be assumed that the magnitude of the hazard ratio corresponds to the quality of the recycled plastic, the following range is set for one grade: $0.1 < (C \times L \times M \times E / T) < 1$ (Equation 1a)

Or, if we consider the hazard ratio for plural plastic chemicals cumulatively,

$0.1 < \sum (C_i \times L_i \times M \times E / T_i) < 1$ (Equation 1b)

where i: individual plastic chemicals.

To separate the portion that depends on the substance from the portion that depends on the usage scenario, Equation 1b is transformed as follows: $0.1 / (M \times E) < \sum (C_i \times L_i / T_i) < 1 / (M \times E)$ (Equation 2)

The value of “ $1 / (M \times E)$ ” will be a separator of each grade.

We obtain the parameters M and E to ensure that the value(s) of “ $1 / (M \times E)$ ” applies to various use cases and exposure scenarios (including exposure route) in plastic products in Japan.

6.08.P-Tu482 The Qualitative Quotient: The Importance of Applying Mixed Methods to Understand Circularity of Hospital Plastics

Nikoline Oturai, Tiffany Ramos, Joy Ndwiga and Kristian Syberg, Roskilde University, Denmark

The healthcare sector, while aiming to protect human health and wellbeing, has a significant negative impact on the climate and environment due to massive resource use and waste production. The Circular Economy, designed to preserve resources in closed-loop value chains, has gained global attention but lacks practical implementation. This study focuses on the challenges of implementing circular economy initiatives for single-use plastics in hospitals, with a specific emphasis on practitioner involvement. The scientific literature on single-use plastics in hospitals primarily uses quantitative methods, such as life cycle assessments and waste audits.

This study suggests that using mixed methods (combining quantitative and qualitative measures) can provide insight into the day-to-day reality of medical staff and their unique perspectives on healthcare practices. This study focuses on the healthcare sector's environmental impact and the implementation of circular economy initiatives for single-use plastics, which directly addresses the 'triple planetary crisis' of pollution, climate change, and biodiversity loss. By integrating social elements and practitioner perspectives, the research highlights the complex interplay between human health, environmental sustainability, and regulatory frameworks. The mixed-methods approach offers a comprehensive understanding of the challenges and opportunities in reducing pollution and resource use in healthcare, contributing to the broader goals of improving EU chemicals legislation and promoting integrated solutions.

The presentation will show preliminary results from a mixed-methods, multi-partner, research project rooted in the circular economy principles, CircleHealth, which seeks to identify barriers to reducing single-use examination gloves at Danish hospitals.

6.08.P-Tu483 A Circular Economy of Chemicals Roadmap for the UK

Miriam Fsadni, Elizabeth Gibson and Matthew Royle, Newcastle University, United Kingdom

Ethylene is one of the most important chemicals manufactured globally, a platform chemical for a vast array of products. The olefins ethylene and propylene account for >70% of all organic chemical production, including polyethyleneglycols via ethylene oxide which are used in cosmetics, pharmaceuticals, lubricants and paints. To defossilise olefin production and support the UK's pledge to achieve net zero carbon emissions by 2050, a pathway to a circular economy of chemicals is needed. The

aim of this research project is to prepare a roadmap, showing the current status and future prospects of the circular economy of olefins.

A whole systems approach is used to identify the challenges and opportunities associated with the transition from a linear to a circular model. This involves looking at the availability of new technologies, and also the business, economic, societal and political landscape. The roadmap draws on consultations with stakeholders, for a holistic view of the chemical industry and related supply chains. These include SME and multinational companies from the pharmaceutical and personal care industries.

Within this framework, the roadmap looks at fostering resource efficiency, reducing carbon emissions, replacing petrochemical feedstocks and reducing waste. This includes: evaluating new and established technologies; identifying products or feedstocks for circular routes; identifying where new standards and testing may be required; as well as understanding where conflict or competition with other sectors may arise. The roadmap also aims to highlight where policy change is planned and needed in order to make the transitions in the required time, and provide a structure on which to compare progress and impact internally and externally.

6.08.P-Tu484 Implications for Implementing the “Essential-Use” Concept in Chemical Regulations
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The Stockholm Convention and the EU REACH Regulation are two key regulations on chemicals at the global and European level, respectively. Discussions have taken place on how to improve them and make them more efficient. For instance, the European Commission is considering implementing the essential-use concept in the REACH Regulation to guide decision-making for phasing-out the uses of the most harmful chemicals. Here we shed light on how the essential-use concept may alter and improve existing regulation by evaluating how existing regulatory outcomes would compare to outcomes derived using the concept and by identifying reasons for discrepancies, including a case study. Overall, this study suggests that existing regulations already consider elements of the concept in their decision-making, and that no drastic changes in the processes are necessary to implement the concept. For the authorities to properly assess the uses of a substance of concern, the industry would need to provide more technical information if they wish to obtain a derogation for their use, similar to the REACH Authorisation process. Further work should investigate what kind of and how much information would be enough to determine whether a use is necessary for health and safety, or is critical for the functioning of society.

6.08.P-Tu485 Ecotoxicological Hazards of Lithium and Its Salts and Regulatory Risk Management Measures

Odile Kerkhof, Stephane Jomini and Cecile Michel, ANSES French Agency for Food, Environmental and Occupational Health & Safety, France

Lithium has geogenic sources, thus it is naturally found in the environment in varying concentrations. Lithium and its salts (lithium carbonate, chloride and hydroxide) are widely used by industries and consumers, in particular in the rechargeable battery sector. Following a classification proposal of the 3 lithium salts (lithium carbonate, chloride and hydroxide) as toxic for the reproduction under the CLP Regulation 1272/2008/EC (on classification, labelling and packaging of substances and mixtures), and considering the growing uses and the new mining projects being developed in Europe, the French Agency for Food, Environmental and Occupational Health and Safety investigated the environmental hazards of lithium and its salts. The objective was to determine whether further regulatory risk management measures are necessary for the environment. The investigation was based on relevant studies from the registration dossiers under the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) regulation and scientific literature. Lithium showed chronic toxicity for three trophic levels (algae, invertebrates and fish) and could meet the criteria for harmonised classification under the CLP Regulation. Lithium also induced developmental defects in several species. Some endocrine disrupting properties were also evidenced. Finally, it induced phytotoxicity in various plant species. There is an urgent need to determine the extent to which human activities contribute to environmental occurrence and effects of lithium. Based on these outcomes, this work highlighted the need to derive an ecotoxicological reference value and to perform risk assessments for its uses throughout the life cycle. Regulatory actions such as e.g. CLP classification, measures under the Water Framework Directive should be considered.

6.08.P-Tu486 D.N.A-based Development of Chemical Accident Prediction and Risk Assessment Technology

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The number of casualties in chemical incidents increase steadily despite the systematic management of chemical substances since implementation of the Chemical Substances Control Act in 2015.

A chemical accident is the uncontrolled and unintentional release of chemical substances, which could potentially result in impair to public health and the environment. Depending of the chemical properties, it can cause even greater damage human and the environment. Although the domestic chemical industry has made rapid growth, it has not followed by safety awareness of chemical substances and preparedness of chemical accident management. In chemical accidents, it is important to strengthen the initial response and establish a systematic prevention system.

This research institute has developed and established models of D.N.A-based chemical accident prediction and risk assessment technology for preventing chemical accidents since April 2022. The two models based on data from government agencies and chemical companies. The chemical accident prediction model utilized data such as the subscription status of employment and industrial accident compensation insurance in Gyeonggi-do, the current status of hazardous chemical handling companies in Gyeonggi-do, and the current status of hazardous chemical accidents in Gyeonggi-do. The chemical accident risk assessment model utilized data provided by the National Institute of Chemical Safety, meteorological data, and NFPA 704 risk codes. We has currently conducted additional data analysis and preprocessing to enhance the current platform, and plan to apply two models to companies in Gunsan-si to verify the effectiveness. Additionally, the causes of chemical accidents are not only technical defects, but also environmental and social factors. Thus, we plan to establish a process prediction model that includes environmental and social factors. This process includes data on SOP, instructions, and maintenance.

The chemical accident prediction platform developed by this research institute can be utilized basic data for policy establishment in related fields in the future, and assist chemical accidents prevention by local governments and companies.

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6.08.P-Tu487 Risk-based Approach for Regulation of Marine Pollution

Kirit Wadhia, NOV, United Kingdom

Introduction: It is apparent that regulations worldwide exist for management of marine pollution dealing with different industry sectors. There is disparity between the different frameworks employed and even within. Criteria for different sectors require particular and specific considerations.

Concern of marine pollution impinges on the change pertaining to physical, chemical, and biological state of the water bodies and coastal areas. The 1982 UN Convention on the Law of the Sea defined marine pollution as: the introduction by man, directly or indirectly, of substances or energy into the marine environment which results or is likely to result in such deleterious effects as harm to living resources and marine life. (UNCLOS, 1982). The most significant threat is the potential impact on marine wildlife and ecosystems.

Chemical pollution in general can emanate from a range of sources including crude oil and other petroleum products, antifoulants, pesticides, pharmaceuticals and personal care products.

In context of the petroleum sector industrial discharge (i.e. produced water) containing chemicals from anthropogenic and natural sources is of primary concern.

The offshore oil and gas industry thematic strategy (Offshore Strategy) sets the objective of preventing and eliminating pollution and taking the necessary measures to protect the OSPAR maritime area against the adverse effects of offshore activities so as to safeguard human health, conserve marine ecosystems and, when practicable, restore marine areas which have been adversely affected.

Risk assessment involves evaluation taking into consideration the potential direct and indirect consequences. It involves comparison of the exposure of the ecosystem to chemicals of pertinence & concern with the sensitivity of the ecosystem for these chemical through the specific exposure-route. Perspective pertaining to the regulatory framework with insight of developments, considerations and progress concerning risk-based approach for the management of produced water discharges is the focus of this conveyance.

Reference

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6.08.P-Tu488 Exposure Based Risk Assessment for Absorbent Hygiene Products

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Disposable absorbent hygiene products (AHPs) (diapers, menstrual products) have made an important contribution to the quality of life and skin health of millions of people. Regulatory classifications vary across countries, regardless of that, human health risk of products needs to be assured with due diligence. This can be achieved through a classical toxicological approach of hazard identification, exposure assessment followed by risk assessment to mitigate any safety concern of intentional as well as unintentional presence of substances. Although the intentional ingredients, which construct the AHPs, are well characterized from a safety perspective, it is the possible presence of trace chemicals that prompts NGOs, regulators and other stakeholders to challenge the product safety for consumers. EDANA volunteered to champion a transparent trust building initiative to self-regulate trace chemicals possibly present in AHPs, using scientific and standardised methodologies. The Stewardship Program CODEX consists in a list of trace chemicals purposely chosen, guidance values for each substance (class) and a standardized test methods (CEN Workshop Agreement 18062) to evaluate products for possible traces of substances. The CODEX values are connected to the respective test methods and the values as such should be decoupled from safety aspects, as they are based on regulatory/standard limits and do not include the aspect of actual human exposure. It needs to be noted that the method is producing very conservative results as it allows for a more intensive extraction of potentially present chemical substances from the product than under normal conditions. Test results based on the CODEX analytical method represent the amount of chemical that is extractable from the product under the experimental conditions. Extractables, based on the CODEX analytical method are not directly comparable to migratable limits that are derived from scientific exposure limits. Detection indicates the mere presence of certain trace chemicals in a complex product matrix like baby diapers or sanitary napkins and is only indicative of a hazard, but not indicative of safety or risk of using these products. Exceeding CODEX limits does not necessarily mean the products are unsafe. Exposure Based Risk Assessment (EBRA) is needed to assess whether the detected concentration of a chemical substance carries any risk from consumer safety perspective.

6.08.P-Tu489 A Tragedy of the Commons – The Case of the Lake Kutubu Indigenous Fishery in Papua New Guinea

Ross Smith, *Hydrobiology, Australia*

Lake Kutubu is the second largest lake in Papua New Guinea, and is a Ramsar Listed Wetland because The Lake's extraordinary level of endemism (10 of the 14 fish species found there are endemic to the lake itself) exceeds that of any other lake in the entire New Guinea-Australian region (RAMSAR 2024 Nov 18). The fishes have been a crucial component of the traditional diet of the Foe People of the lake, and they have had both a traditional custodian role as well as a legally recognised role in management of the lake ecosystem since the lake was identified as a Wildlife Management Area under the Fauna (Protection and Control) Act. However, there are increasing concerns regarding the effects on the fishes and the fishery of the development of an oil and gas industry in the region, and the associated increase in road links and regular air services to the lake catchment.

Although there have been claims of direct impact of the petroleum industry on the lake (Smith et al. 2015; Schneider et al. 2016; Elapa 2024 Oct 16), the Foe are also well aware of the impacts of their own increasing population growth and increased access to modern fishing technologies on the fishery. The introduction of exotic fishes into the lake has also complicated the management of the native fisheries, even though a method to eradicate one of the exotic species has been suggested (Thresher et al. 2020). This paper will summarise the circumstances that have contributed to the current challenges to manage the fishery and the lake ecosystem, including collaboration with the Foe people over the last 15 years to better understand the pressures and stressors affecting the lake.

Disclaimer/Disclosure: The author thanks the Foe People for their friendship and support and the financial and technical support of Oil Search, SANTOS, the Conservation and Environmental Protection Authority and the National Fisheries Authority over the course of 15 years of study of Lake Kutubu.

6.08.P-Tu490 Revitalizing Indigenous Knowledge in Global Climate Science and Policy Processes

Beatrice Olutoyin Opeolu¹ and **Linda Sibali²**, (1)BEE Solutions and Consultancy Services, South Africa, (2)University of South Africa, South Africa

For over 350 million indigenous peoples worldwide, climate change impacts are expected to be early and severe due to their location in high risk environments. Climate change impacts pose existential threat to millions of indigenous peoples across the globe. Indigenous knowledge systems are critical to the achievement of SDG 13 (Climate Action). Climate change effects include heat waves, severe storms,

drought and diseases with consequent implications on ecosystems, human health and socio-economic systems. Major drivers of climate change are resource utilization-driven activities. These are exacerbated in the developing world by lack of infrastructure, poor governance and poverty. Issues of water scarcity, biodiversity loss, loss of livelihoods due to extreme weather events continue to negatively impact global ecological and human health systems. A paradigm shifts in practices to incorporate indigenous knowledge into production systems, management and governance of natural resources is now critical for a sustainable world. Agriculture, medicine and natural resources management require holistic approaches for a sustained future. Insights into some of these issues and possible mechanisms for effective stakeholder engagement for tangible impacts in the context of a developing country will be provided.

6.08.P-Tu491 Energy Efficiency, Effectiveness, and Derivatives Assessment in the Crop Agricultural Sector: Parametric and Non-Parametric Methods Application Review

Hamza Taoumi and Khadija Lahrech, Sidi Mohamed Ben Abdellah University (USMBA), Morocco

Energy scarcity and natural resource availability are the main challenges facing crop agricultural researchers and scholars worldwide. Consequently, searching for their optimization becomes an essential path to follow to guide policymakers in establishing strong strategies aiming to attain sustainability. Several methods exist in the literature to choose among the practices to apply for energy and resource consumption optimization in the crop agricultural sector. Two essential methods were the subject of an updated and critical review of the crop agricultural research articles contributions to the energy performance evaluation according to their mapping, their functions, their aspect, and the energy variable occupation degree: The Data Envelopment Analysis, and the Stochastic Frontier Analysis. Based on peer-reviewed research recorded from 2019 to 2023, 28 original articles existing in the literature were gathered from the databases Scopus and Web of Science to dig deeply into the energy efficiency, effectiveness, and derivatives evaluation through the DEA and SFA application. The analysis showed that those methods are the basic methods used for energy performance evaluation, and the energy occupies a significant role as a primary variable to be included in the optimization problem solving as an input, output, or influence factor to follow during different ecosystem stages. Thanks to the DEA and SFA multidimensional consideration of different aspects and variables in the optimizing equation, it could be concluded that the energy variable is a primary path among others to follow during the entire stages of the ecosystem evaluation. Such an approach would help policymakers define strategies to obtain more goods while focusing on energy consumption and wastes, economic costs, environmental damages, and social impacts.

6.09.P Almost there? Latest News on Pollinator Risk Assessment in the EU

6.09.P-We508 Bee Risk Assessment in the Real Regulatory World

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The EFSA Bee Guidance Document (EFSA Bee GD, 2013) significantly updated the simple approach of the prior guidance (SANCO/10329/2002 rev 2, 2002). In 2023, EFSA published the revised Bee GD. The risk assessment scheme has increased in complexity by integrating various exposure pathways into a comprehensive evaluation of their overall impact on colony health. At the time of abstract submission (November 2024) this guidance is not adopted or implemented for use in the European Union pesticide regulatory procedure. Amongst stakeholders, there is no agreement on whether to follow EFSA's recommendations and several alternative approaches are in place.

All the aforementioned guidance covers only chemical plant protection products (PPPs) and lacks directives for addressing non-chemical PPPs like microbials and natural substances. Such substances play an important role for the target to reduce the use and related risk of chemical pesticides as well as the use of the more hazardous pesticides by 50% by 2030.

Currently, there is no harmonised approach on bee risk assessment. Perspective on a timeline for implementation of the revised EFSA Bee GD is still lacking, leading to a high level of uncertainty for applicants as well as for evaluators who work for national authorities.

During this transition period member states and regulatory zones defined their own strategies. There are several national approaches either following an in-between solution or specific national or zonal approaches, such as the Northern Zone approach including a specific calculator tool for chronic bee risk assessment.

Further challenges are emerging for biopesticides and naturally occurring substances, which often do not fit into the classical risk assessment and testing scheme used for chemical PPPs.

For microbial substances and PPPs, specific data requirements are now in place, but there is still a lack of agreed test guidelines and approaches how to address infectivity/pathogenicity in the different test systems.

To meet the goals set for the reduction of the use of PPPs, application techniques such as spot or precision

application are supported. However, there is no consistent guidance for how to model or reflect these approaches in risk assessment calculations or in the performance of higher Tier bee studies. The presentation will share the perspective of a regulatory consultant on the current bee risk assessments as evaluated by various member states for different substance types.

6.09.P-We509 First Practical Experiences with Equivalence Testing in Pesticide Risk Assessment for Bees

Silvio Knaebe¹, Pierre Mack² and Holger Bargaen², (1)Eurofins Agrosience Services US, Germany, (2)Eurofins Ecotox, Germany

Equivalence testing was first introduced in the context of pesticide risk assessment in the new draft guidance document for the risk assessment of pollinators (EFSA 2023). The methodology is already used in clinical trials to compare different formulations of the same group. It is a statistical method that aims to prove the safety of a substance by demonstrating that any potential effects are smaller than the predefined acceptable threshold of 10% (?).

Equivalence testing contrasts with traditional difference testing, which aims to detect statistically significant differences between a treatment and a control group, often leading to inconclusive results regarding safety. In contrast the equivalence testing directly addresses the safety of a substance by aiming to prove the absence of relevant effects. Additionally, it allows researchers to directly control the risk of failing to detect a high-risk substance (false-positive rate). This error is of primary concern to risk assessors. The outcome of an equivalence test directly informs the conclusion regarding high or low risk, unlike difference testing which may lead to inconclusive results.

A further key advantage according to EFSA is that the study design can adapt to the level of concern associated with the plant protection products (PPP). No specific replication level is mandated, power analysis can inform the applicant about the likelihood of proving safety given specific effect size assumptions. Studies of high-concern PPPs may require more resources (e.g., replications) than those of low-concern PPPs.

This data explores the application of equivalence testing with data based on a real study. Using a mock dataset based on real field studies with a low-concern pesticide, we investigated the feasibility of equivalence testing with in vivo data from honeybee colonies. Our analysis included assessing colony strength across various field pair combinations and time points. Results demonstrate the potential of equivalence testing to effectively evaluate pesticide safety, even with the high natural variability observed in bee colonies. Notably, our findings suggest that the required number of field pairs for robust assessment may be lower than current EFSA recommendations, highlighting the potential for resource optimization in risk assessment studies.

6.09.P-We510 Learnings from a Failed Meta-Analysis: Sub-Lethal Effects of Plant Protection Products on Bees - A Case Study

Tobias Pamminger¹, Magdalena M. Mair² and Christian Maus¹, (1)Bayer AG, Germany, (2) University of Bayreuth, Germany

Chemical plant protection products are designed to manage pests, weeds and disease in a wide range of crop plants ensuring stable yields. While effective in limiting pest populations they can come in contact with non-target organisms including bees. Such unintended exposure can result in a range of side effects including, among others, sub lethal ones such as altering movement, learning and feeding behavior. While the existence of such effects is well documented in bees, there have only been a handful of attempts to quantitatively assess their occurrence across products and species. In this study we attempted to fill this gap by performing a formal meta-analysis on the occurrence, strength, and potential dose dependency (sequence of effects) of sub lethal effects in adult bees moving beyond a simple analysis of their existence. We find an extensive bias in the existing data both in terms of product x species tested with the majority of studies focusing on insecticides targeting the nicotinic acetylcholine receptor (e.g. Imidacloprid and Clothianidin) in the honeybee. In addition, we find that a large proportion of the studies do not adequately report the necessary methodological details and results to reliably extract the statistical information necessary to conduct a formal meta-analysis across chemical plant protection and the different types of sub lethal effects. We discuss the cause and consequences of these findings for studying sub lethal effects in bees beyond their existence and provide recommendations to improve the reporting standards of such studies.

Disclaimer/Disclosure: TP and CM work for the Bayer AG a manufacturer of plant protection products.

6.09.P-We511 Effect Models for Higher-Tier Risk Assessments: The Example of SolBeePop_ecotox, a Population Model for Solitary Bees

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In the revised bee guidance document released by EFSA (2023), ecological effect models are recognized as useful tools to support higher-tier pesticide risk assessments for bees. Models can be applied to support study data and extrapolate from tested to untested situations, e.g., differing environmental conditions. For solitary bees, models additionally could be used to extrapolate to untested species given the large diversity of species and ecologies. Natural as well as managed populations could be addressed. With the population model for solitary bees, SolBeePopecotox, we demonstrate the application of a model that can be applied in support of higher-tier risk assessments. The model can be used to simulate multiple solitary bee species, using their species-specific ecological traits as parameters. Exposure routes are considered explicitly, including direct spray, residues in pollen and nectar as well as in nesting materials. Effects are implemented in the model using a simplified toxicokinetic-toxicodynamic model, BeeGUTS, adapted specifically for bees. The performance of the model was demonstrated using available data from semi-field studies with the red mason bee, *Osmia bicornis* L., simulating bees foraging in tunnels over control and insecticide-treated oilseed rape. In addition, we applied the model to simulate hypothetical semi-field studies with untested soil-nesting solitary bees species, the alkali bee, *Nomia melanderi* COCK., and the hoary squash bee, *Eucera pruinosa* SAY. With this example of a population model for solitary bees, we demonstrate how models could be evaluated and analysed, and how their applications could support higher-tier risk assessments of bees. Evaluation and applications of ecological models can provide insights into their acceptability for use in pesticide risk assessments, supporting the pathway to become standard tools in risk assessments as envisioned by the EFSA guidance document as well as other regulatory frameworks.

6.09.P-We512 Bumble-BEEHAVEecotox – A Mechanistic Effect Model for Bumblebees

Dominik Lammers, Thomas G. Preuss and Vanessa Roeben, Bayer AG, Germany

Pollinators, particularly bumblebees, are essential for ecosystem health and agricultural productivity. However, understanding the impacts of various stressors, including pesticides, on bumblebee colonies presents significant challenges for environmental risk assessments. This study aims to develop a framework for Bumble-BEEHAVEecotox, an ecotoxicological module for the Bumble-BEEHAVE model, to predict effects at the colony level based on laboratory data from individual standardized studies.

The Bumble-BEEHAVEecotox framework will be built upon the existing BEEHAVEecotox template and will proceed in two main steps. First, we will implement the capability to simulate tunnel studies, which will involve accurately defining landscape parameters and setting up bumblebee colonies at specific locations with precise timing. This will allow us to judge Bumble-BEEHAVEs ability to simulate bumblebee colony dynamics. The second step will integrate two effect modules: one based on the dose-response relationship used in BEEHAVEecotox and another utilizing the General Unified Threshold model of Survival (GUTS).

Our model will be validated through semi-field studies, starting with control studies to evaluate baseline colony dynamics, followed by studies that incorporate plant protection products to compare predicted effects with empirical data.

Bumble-BEEHAVEecotox represents a significant advancement in understanding the impacts of environmental stressors on bumblebee populations. By enabling comparisons of various stressors, including landscape changes and pesticide applications, this modular modeling framework can inform more effective risk management strategies and contribute to the conservation of pollinator populations in changing landscapes.

6.09.P-We513 Simulations of Population-Level Effects of Pesticides on Abundance and Pollination of Bumble Bees in Landscapes of Different Composition Allow the Identification of Critical Application Levels

Andreas Focks¹, Leonhard Urs Bürger¹, Steven Droge², Daniel Paredes Llanes³, Jose Paulo Sousa³, Grzegorz Sylwester Sowa⁴, Louise Wipfler² and Lorraine Maltby⁴, (1) Osnabrück University, Germany, (2) Wageningen UR, Netherlands, (3) University of Coimbra, Portugal, (4) University of Sheffield, United Kingdom

This study aims to enhance the environmental assessment of non-target organisms (NTAs) concerning plant protection products (PPPs) by linking individual impacts to effects on ecosystem functions. Simulation models that analyze the population dynamics of sensitive species, like non-managed

bumblebees in agricultural settings can create such links. Specifically, we examine the effects of a hypothetical pesticide on bumblebee populations and their pollination function across landscapes to establish critical application rates that affect bumblebee populations and pollination services. Bumble-BEEHAVE is an individual-based model designed to analyze bumblebee foraging behavior based on floral resource availability and environmental factors. Implemented in the BEE-STEWARD software using NetLogo, the model was adapted for this study by incorporating a pesticide module that simulates the pesticide's fate and effects. The model accounts for pesticide emissions, decay, and calculates the increased mortality risk for foraging bumblebees using a log-logistic dose-response function. We simulated *Bombus terrestris* exposure in a 25 km² agricultural landscape in South England (Fowey) after a hypothetical pesticide application on day 90 with various initial concentrations ranging from 0.1 to 100 µg/m² and a derived DT50 of 20 days and an LC50 of 2.4 µg/m². Simulations illustrated the negative impact of increasing pesticide concentrations on bumblebee abundance and pollination performance. As pesticide concentrations increased, both bumblebee abundance and foraging visits decreased. A dose-response analysis highlighted the possibility to derive critical concentration thresholds necessary to restrict bumblebee population inhibition to under 10%. Furthermore, action at a distance was observed, with pollination in non-exposed natural areas diminishing at intermediate concentrations of the pesticide. This research demonstrates the utility of simulation models in linking individual-level effects of PPPs to broader ecosystem functions, considering ecological traits, chemical properties of pollutants, and landscape dynamics. Through these simulations, critical thresholds for pesticide application levels can be established to protect vital ecosystem services like pollination.

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6.09.P-We514 B-Risk, A Calculator Tool Implementing the Latest EFSA Bee Guidance Document
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EFSA has reviewed its guidance document (EFSA, 2013) on the risk assessment of plant protection products and bees (*Apis mellifera*, *Bombus* spp. and solitary bees). The reviewed guidance document, published in 2023, outlines a tiered approach for exposure estimation for different scenarios, it includes updated hazard characterisation and provides risk assessment methodology for all bee groups. Performing lower tier risk assessments according to this guidance requires complex exposure estimations for the different scenarios and routes of exposure, fitting dose-response curves for different risk cases and combining the exposure and hazard estimations to predict the colony or population level effects. In addition, several specific aspects covered in the guidance (e.g. time reinforced toxicity, mixture toxicity) require some computations.

In light of these complexities, the creation of a calculator tool was deemed necessary by EFSA. The tool is available as a Shiny app on the EFSA R4EU platform. It will also be made available in the future for all interested stakeholders in the Knowledge Junction repository as an R package which could be also used locally for knowledgeable R users. A first testing phase of the tool was performed in the first half of 2024 with the involvement of several member state experts and stakeholders representing chemical industry. The tool was published in early November 2024 and is accompanied by a detailed manual. The poster will explain the main modules of the tool and will illustrate some functionalities.

6.09.P-We515 BeeGUTS R Package, Mechanistic Modelling of Bee Survival for Environmental Risk Assessment Following EFSA guidelines

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The use of GUTS modelling for the analysis of survival data has been recognized as a powerful tool for environmental risk assessment. Recently, the BeeGUTS model has successfully opened the possibility to analyze all the laboratory tests done on adult bees (acute and chronic) within a single mechanistic TKTD framework, allowing to derive a single set of test-independent GUTS parameters. The BeeGUTS R package implements this method in an open-source, user-friendly tool. The calibration of the model parameters is performed using Bayesian inference through the robust Stan platform. With the package, it is possible to perform all the steps described by EFSA for calibration and validation of a GUTS model. The most recent update of the package introduces the tools for calculations required by the EFSA guidance on bees, such as the time reinforced toxicity test, and it expands the usability for the various steps of the risk assessment process using TKTD modeling.

6.09.P-We516 GUTS for Bee TRT Assessment: A Methodology Comparison

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The revised EFSA guidance on the risk assessment of pesticides on bees, likely becoming effective in 2025, requires the assessment whether a substance exhibits Time-Reinforced Toxicity (TRT) properties. TRT of a substance is indicated if exposure to low doses over long time periods causes higher effects in organisms than higher exposure over short periods. For this assessment, the reduced versions of the General Unified Threshold Models of Survival (GUTS-RED) are employed. Thereby, two versions of the model, using the assumptions of stochastic deaths (GUTS-RED-SD) or individual tolerance (GUTS-RED-IT), are calibrated to data from an OECD 245 chronic oral toxicity study on adult honey bees, and the LDD50 is estimated for 27 days, i.e., for the life span of summer bees. This calculation step is included in the risk calculator tool under development by EFSA. However, there are multiple other software implementations of GUTS models openly available, including multiple R packages and openGUTS. We used several alternative software options available for GUTS to calibrate to hypothetical chronic study data. We compared the predicted LDD50s and resulting conclusions of the TRT hazard assessment. If similar, the comparison can improve confidence in model outputs. Discrepancies might point to issues with calibration data or software implementations. We think this approach is an important step in the reliable application of GUTS-RED in lower-tier risk assessments of bees.

6.09.P-We517 Introduction of a New Interest Group for Bee Modelling in the Context of ICPPR

Amelie Schmolke¹, Jeffrey Minucci², Vanessa Roeben³ and Johannes Luckmann¹, (1)RIFCON GmbH, Germany, (2)US EPA Office of Research and Development, United States, (3)Bayer AG, Germany

Mechanistic effect models have been identified as relevant tools in the context of risk assessments of bees and other pollinators in guidance documents in the EU and North America. Several models have been introduced for the purpose or are under development, spanning the representation of organism-level effects (GUTS), honey bee colony models and population models of non-Apis bees. In the context of the International Commission for Plant-Pollinator Relationships (ICPPR), an interest group for bee modelling was established in October 2024 with the aim of improving the acceptability and usability of effect models in bee risk assessments. The interest group provides a platform for exchange and discussions between stakeholders from different organisations including regulators, industry, CROs and academia as well as different professional backgrounds such as modellers, policy makers and bee experts. The interest group has the goal of identifying important steps that can improve model applications and support acceptability of models for risk assessments. Manageable projects relating to the identified steps will be conducted in the context of the interest group. We are presenting this effort at SETAC to interconnect the two communities and bolster communication.

Disclaimer/Disclosure: This work is not a product of the United States Government or the United States Environmental Protection Agency, and the author (Jeffrey Minucci) is not doing this work in any governmental capacity. The views expressed are those of the author only and do not necessarily represent those of the United States or the US EPA. In general, the views expressed in this presentation and by the interest group do not necessarily represent those of the authors' or members' affiliations.

6.09.P-We518 Integrating Landscape-Scale Simulations with TKTD Models for Pollinator Risk Assessment

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Landscape-scale simulations are gaining prominence in environmental risk assessment as comprehensive tools for evaluating the impacts of chemicals in complex ecosystems. Toxicokinetic-toxicodynamic (TKTD) models, like the general unified threshold model for survival (GUTS), are well-established for assessing toxic effects under realistic exposure scenarios but have limitations when applied to spatially explicit, individual-based models. These challenges include solving differential equations for each agent at every timestep and addressing species-specific adaptations for multiple uptake routes, common in terrestrial organisms.

Here, we present the integration of the "buffer GUTS" model, a generic TKTD framework capable of handling multiple uptake routes without requiring species-specific information. This model is calibrated to parameterize the relative contributions of each uptake route to the overall toxic effect. We achieve a computationally efficient solution by discretizing buffer GUTS into algebraic equations for state variables at each timestep. Additionally, we address the damage threshold problem by introducing an extra timestep when damage crosses the threshold within a single step, ensuring precision equivalence to ODE solutions. Our approach describes the buffer GUTS for oral, topical and contact uptake routes, and we demonstrate

its application using the *Osmia bicornis* model from the Animal Landscape and Man Simulation System (ALMaSS) as a case study. This integration represents a scalable and precise method for coupling TKTD models with landscape-scale simulations, broadening the scope of environmental risk assessments to terrestrial and multi-route exposure scenarios.

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6.09.P-We519 Integrating Allometric Scaling in Solitary Bee Risk Assessment: A Mechanistic Approach

Vanessa Roeben¹, Zhenglei Gao¹, Mark Miles², Tobias Pamminer¹ and Thomas G. Preuss¹, (1)Bayer AG, Germany, (2)Bayer AG, United Kingdom

Solitary bee risk assessment has been a challenge for a decade. Globally there is currently no agreed framework and testing strategy on how to conduct a solitary bee risk assessment. In the recent draft of the revised bee guidance for Europe, EFSA proposed so called Toxicity extrapolation factors (Tefs) to cover solitary bees based on honeybee data for the environmental risk assessment.

In the preparation for the new guidance, EFSA has done a rigorous job to collect data, evidence, and knowledge of solitary bees and have identified allometric scaling as a useful component for the bee risk assessment. With this work we take a logical next step to include allometric scaling in a scalable and flexible mechanistic risk assessment framework, based on the great data and knowledge collection of EFSA.

Our study will showcase that using honey bee as a surrogate for intrinsic sensitivity is protective for other bee species, based on the critical body burden approach. By integrating allometric scaling into the remaining parts of the risk assessment, we are separating sensitivity, exposure and vulnerability which allows us to use the honey bee as a surrogate for sensitivity and include exposure and vulnerability by simple equations or realistic models. Furthermore, we highlight the importance of energetic needs and protein consumption in reproductive health, linking these factors to body size and exposure levels. With the presented framework, we are reducing the complexity of solitary bee risk assessment by simplifying the approach based on sound scientific knowledge without additional experiments and thus enabling tailored risk assessments for locally important species.

Our findings contribute to the ongoing dialogue surrounding the EFSA bee guidance by proposing a structured methodology for risk assessment that is applicable across various geographical contexts.

6.09.P-We520 Realistic Field Exposure - Pilot Study on Refining Bee Exposure Residue Data: Comparative Analysis of Field and Tunnel Studies for Phacelia Crops

Silvio Knaebe¹, Pierre Mack² and Farnaz Faramarzi², (1)Eurofins Agroscience Services US, Germany, (2)Eurofins Ecotox, Germany

Bees are essential pollinators, and it's crucial to ensure that pesticides used in agriculture don't harm them. Currently, pesticide risk assessments for bees often rely on "worst-case scenario" data, which may overestimate the actual risk. To address this, the European Food Safety Authority (EFSA) has proposed new guidelines (EFSA2023) that allow for more realistic exposure estimates.

EFSA2023 outlines two main approaches for conducting bee field studies:

1. Minimum Alternative Forage Studies: These studies aim to create a worst-case exposure scenario by ensuring that less than 10% of the landscape within a 4 km radius of the study fields contains alternative forage resources. This setup ensures bees forage almost exclusively on the treated crop, maximizing residue exposure measurements in pollen and nectar.
2. Randomly Selected Landscape Studies: These studies are designed to represent realistic field conditions by selecting 15 locations across the intended area of pesticide use. Bees are exposed to treated crops within diverse landscapes, including natural forage, to measure residues under typical foraging conditions while maintaining statistical reliability for residue data

Despite this proposal as a refinement option, these approaches have not yet been implemented in practice. To gain some practical aspects and estimate realistic reductions in residues when transitioning from worst-case tunnel data to real-life field conditions we conducted a small Pilot Project

Our study compares residue data in pollen from two distinct settings:

- Field Set-up: Residues were measured at four Phacelia field sites.
- Tunnel Set-up: Residues were measured at two enclosed Phacelia tunnels.

All sites were applied at the same rate of a commercial pesticide and samplings took place at 3 different sampling intervals at 0, 1 and 3 days after the respective application. In total, we generated 10 residue values in the field and 6 values in the tunnels.

Residues in the field were reduced by more than 80% compared to the tunnel.

This comparative analysis aims to provide critical insights into the refinement of residue data for more accurate risk assessments for bees.

6.09.P-We521 A Case Study of Assessing Sublethal Effects to Honey Bees For A New Insecticide Considering the EFSA Revised Bee Guidance

Charlotte Elston¹ and **Annette Kling²**, (1)*Syngenta, United Kingdom*, (2)*Eurofins, Germany*

Due to growing concerns and specific calls for greater consideration of sublethal effects to protect bee populations, the EFSA revised Bee Guidance document (2023) suggests assessing sublethal effects of PPPs as part of the effect-tier assessment. The rationale for this is that large scale behavioural changes may interfere with important tasks, such as foraging, which have the potential to translate to colony level effects and hence the Specific Protection Goal (SPG) of colony strength. Behavioural abnormalities are quantitatively observed according to given categories in the OECD 245 honeybee adult chronic test guideline (moribund, affected, cramps, apathetic, vomiting). Some barriers to developing a more comprehensive assessment of sublethal effects include lack of standardisation, lack of proven link to the SPG of colony strength, subjectiveness of visual assessments and potential for unconscious bias. We present a 10 day adult honeybee chronic study conducted according to OECD 245 with a new insecticide in development which included blind assessments of sublethal effects and mortality, where the treatment groups were not known to the assessors.

In the control and carrier control, no behavioural abnormalities were observed throughout the test period. In the treatment groups, behavioural abnormalities (affected bees, moribund bees, cramping bees) were observed in all test item treatment groups, at all assessments, throughout the entire observation period but was not dose dependant. Results from the blind assessments correlated well with the range-finder test which was not conducted blind, indicating that the current test methods for assessing sublethal effects are robust within the same laboratory (between different assessors in the same laboratory). However, categories of behavioural effects given in the test guideline are open to interpretation as with any visual assessment, and adding additional assessments could result in lack of standardisation between studies and a deviation from the test guidelines. Results generated in the 10 day laboratory feeding study were compared to higher tier studies with Apis and Non-Apis bees with this new insecticide in development and discussed.

6.09.P-We522 Toxicity Based Classification System for Bees: A Preliminary Study from Anses

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The environmental classification of active substances, plant protection products and biocides is important information for hazard identification and communication. Whereas harmonised criteria exist for the aquatic environment, such harmonised hazard classification system is not available for other organisms.

For bees, hazard classification approaches are implemented in some European member states (e.g Sweden for Biocidal Products) and in countries outside EU (e.g Canada and USA for Plant Protection Products), sharing some similar criteria. The principle is to consider an acute toxicity endpoint (LD50) greater than 11 µg/bee as a trigger for classification of hazard to bees. This means that below this trigger of 11 µg/bee, a substance or a product containing the substance would be considered toxic for bees and a specific hazard pictogram implemented.

On the basis of data available in the European list of endpoints for active substances in plant protection products and biocidal products, our study aims to identify the type of active substances that could be considered as toxic to bees when considering the above trigger of 11 µg/bee. A focus will be made on acute toxicity data as these are basis of the proposed trigger proposed above for the hazard classification.

6.09.P-We523 Sensitivity Study Using the Imidacloprid Neocotinoid Toxicity Test for Native Bees of the Species *Tetragonisca angustula* (jatai)

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Native bees play a fundamental role in pollination, contributing to reproducing approximately 90% of flowering plant species and 80% of economically important crops. However, pesticide contamination threatens this species' survival, resulting in reduced biodiversity in ecosystems and food supply. Therefore,

it was necessary to determine safe doses of pesticides, such as imidacloprid, to ensure that the bee's function and survival are not compromised. The aim of this study was to determine the lethal concentration of the neocotinoid imidacloprid (commercial product) (LC50) and its lethal dose (LD50) for the jatai bee species (*Tetragonisca angustula*). To determine sensitivity through the acute toxicity test to exposure to this pesticide, adult bees were collected in the early hours of the morning and taken to the laboratory for the start of the toxicity test. The test which was carried out over a period of 6; 24; 48; 72 and 96 hours. The bees were kept under acclimatization in an incubator at a temperature of 28 °C, with food being replenished at all times during the test. The results of the study showed that imidacloprid has a considerable toxic effect on bees from 24 hours of exposure. During this period, the CL50 (1.0333 mg L⁻¹) and DL50 (1.00271 ng i.a./bee) were determined for the commercial product, imidacloprid. It was observed that for imidacloprid at concentration of 0.8 mg L⁻¹ the mortality rate was 55% in the first 24 hours, reaching 67% mortality during the 96-hour period, and for the highest concentration of 1.6 mg L⁻¹ in the first 24 hours of the test the mortality rate reached 100%.

6.09.P-We524 Developments in the Biocides Risk Assessment for Bees

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Until recently there has been no quantitative risk assessment of bees when exposed to biocides. This is in contrast to the risk assessment for pesticides, where the assessment of bees is performed in accordance with SANCO (2002). In addition, extensive updates of the pesticide assessment have been proposed in the form of the updated bee guidance EFSA (2013) and EFSA (2023).

In 2019 the European Chemicals Agency (ECHA) was mandated by the European Commission (EC) to develop guidance for assessing the risks to arthropod pollinators (including bees), and to specify the information required to enable a conclusion to be drawn by any evaluating authority. The result of this is new guidance on how to assess biocide risks to bees (ECHA, 2024). The guidance is very closely linked to the EFSA (2023) guidance, though with some notable differences primarily relating to the estimation of exposure from biocidal uses. In March 2024 ECHA hosted a webinar to introduce the guidance and provide a Q&A opportunity. The new guidance will apply to active substance approvals and biocidal product authorisations for which applications are submitted on or after 1 February 2026.

In this poster the key developments in the bee risk assessment for biocides will be summarised. This will cover: which PT types require an assessment for the risk to bees; the Specific Protection Goals (SPG); Data requirements; emission scenarios (exposure assessment); and the risk assessment itself. The implications of this guidance on biocidal product applications will be discussed.

6.10 From Nanomaterials to Advanced Materials: Challenges and Progress in Research, Industrial Application and Regulation

6.10.T-01 Integrated Approaches to Testing and Assessment (IATA) for MXenes MAX Phases and Advanced Carbon Nanomaterials

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MXenes are two-dimensional (2D) nanomaterials made of transition metal carbides, nitrides, or carbonitrides, derived from MAX phases, which are layered ceramics with alternating layers of transition metals, carbon or nitrogen, and another element like aluminum or silicon. By selectively etching the 'A' element (typically aluminum) from MAX phases, ultra-thin, flexible MXene nanosheets are created. These sheets have high surface area, excellent electrical conductivity, and tunable surface chemistry, allowing modification through various functional groups.

In the SAFARI project, MXenes are being developed for industrial applications in conductive inks, glucose/lactate biosensors, and EMI shielding paints, all aligned with the European Commission's Safe and Sustainable by Design (SSbD) Framework.

The SAFARI project is developing an Integrated Approach to Testing and Assessment (IATA) to evaluate the risks and hazards of both MXenes and their precursor MAX phases. This approach combines in silico tools, in chemico studies, and in vitro methods, using New Approach Methodologies (NAMs) to assess the safety of these nanomaterials. The goal of the IATA is to create a roadmap for evaluating the potential environmental and health impacts of MXenes, ensuring their safety and sustainability.

The first step of the IATA focuses on defining the problem, assessing the risks, and identifying hazardous

properties of MXenes and their production process. The second phase involves gathering existing data on exposure routes, toxicological and ecotoxicological profiles, and physicochemical properties from literature and regulatory reviews. In the third phase, the collected information will be evaluated to identify any gaps. In the fourth phase, toxicological and ecotoxicological tests, workplace monitoring studies, and physicochemical analysis will be conducted. A life cycle analysis will assess the environmental impact of MXenes. If data is insufficient to meet regulatory requirements, further testing will be performed, completing the risk analysis and identification of hazardous properties.

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6.10.T-02 Nanomaterials Risk Assessment - A Regulatory Framework for Assessing the Variability of Nanoforms

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The assessment of nanomaterials poses challenges due to the variability in nanoforms (NFs), which, despite sharing chemical composition, differ in key characteristics like size, shape, and coating. Traditional chemical-based assessments fall short of addressing these complexities. The ECHA-funded project, "Nanomaterial Risk Assessment: A Regulatory Way Forward for Sameness and Grouping Approaches," seeks to create a regulatory framework that efficiently accounts for NF variability while ensuring comprehensive hazard evaluation and regulatory compliance.

Stage one of this project produced a draft framework for assessing sets of nanoforms using decision trees based on key NanoApp characterisers such as composition, size distribution, surface functionalisation, shape, and crystallinity. This phase included a thorough review of over 1,000 publications, analysis of 10 databases, and assessment of 20 validation techniques focused on REACH's Annex VI characterisers. The study highlighted existing tools, such as DF4nanoGrouping, NanoApp, and GRACIOUS, examining their effectiveness and limitations in defining sameness thresholds. The NanoApp emerged as a critical tool, suggesting that tiered assessments could minimise unnecessary testing when characteriser variations stay within specified limits.

However, further exploration is needed to refine thresholds and integrate environmental factors. The project's next stage will validate the proposed framework through case studies and stakeholder-engaged data collection, ensuring robustness and filling critical data gaps with comprehensive nanoform testing.

6.10.T-03 Occupational Safety and Health Profits from a Safe Design

Rolf Packroff and Michaela Clever, BAuA - Federal Institute for Occupational Safety and Health, Germany

The green and digital transformation is accompanied by a profound change in workplaces and working conditions. In line with the UN's global sustainability goals, green workplaces should be designed in a humane way. With this aim, the European Commission's 2022 Joint Research Center has published the concept of safe and sustainable by design of chemicals and materials (SSbD). In this introductory lecture, examples from development of fibrous materials should demonstrate how safe material design has grown historically. Conclusions for future development will be drawn.

It lasted until the seventies to understand that the causes for asbestos related cancers lie within their fibre morphology and biopersistence in the deep lung. This makes clear that other fibrous materials, e. g. mineral wools, ceramic or microglass fibres and whiskers could also pose a cancer risk. But the "fibre principle" also offers the opportunity for a safe design of new fibrous materials, the introduction of biosoluble mineral wools in the late nineties was a breakthrough for the profit of workers health.

Today sustainable development is driving the development and use of fibre materials. Carbon fibres play an important role in lightweight applications, Carbon nanotubes are essential for energy storage systems. But for both classes of materials specific types have been identified as a risk to workers health during particle-releasing tasks. But, also criteria for a safe design were derived from this experimental results and physical considerations. It was confirmed, that rigidity of critical fibres plays an important role in frustrated phagocytosis, which was not proven for extremely thin single carbon nano fibres with diameters in the range below 30 nanometer. For carbon fibres we observe the phenomenon, that only crystalline types with a high density lead to a significant release of critical fibre dusts at the workplace.

We have underpinned the SSbD concept with an approach for safe-to-use chemicals, materials and

products that includes also low-emission forms and safe processes beyond the "golden standard" of avoiding hazardous properties. In addition, risk research on nanomaterials has clearly shown that the development of new materials and material classes must be accompanied by the development of adapted methods for risk assessment and evaluation. Regulation must keep pace with innovation!

6.10.T-04 Improving the Uncertainty Evaluation for Nanomaterial Worker Exposure Assessment for SSbD

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One aspect within Safe and Sustainable by Design (SSbD) frameworks for nanomaterials (NMs) and nano-enabled products (NEPs) that needs to be considered is worker exposure. This is often supported by the use of exposure models or tools. With the use of these tools and models, there can be considerable uncertainty in their outputs. Within control banding (CB) tools for NMs, there are potential improvements and streamlining that could be performed for uncertainty analysis.

We present methodology that has been developed for assessing uncertainty using Theory of Scales of Measurement and the GUM (Guide to the expression of uncertainty in measurement) for worker exposure in NMs CB tools. This methodology consists of five steps. These steps are: 1) Defining the output quantity (i.e. the potential exposure); 2) Identifying the input quantities and their scale (nominal, ordinal, interval, ratio); 3) Defining the allowable basic operations and permissible statistics. At this stage, potential improvements can be added if it is not possible to define the uncertainty contributions; 4) Checking the mathematical model relating output quantity (i.e. the potential exposure) to the input quantities for any mathematical flaw for the scales of the input parameters. At this stage, potential improvements can be added; and 5) Accounting for possible improvements suggested in step 3.

This methodology has been demonstrated using a thermoplastic industrial case study on single-walled carbon nanotubes (SWCNTs) and a polycarbonate (PC) matrix in the CB tool, Stoffenmanager Nano. Within Stoffenmanager Nano there are potential improvements to uncertainty, such as quantifying the overall uncertainty. Using this case study, we have assessed the input parameters (such as handling of NMs) and the probability distribution using the exposure algorithm in Stoffenmanager Nano to identify potential improvements to the uncertainty assessment.

Our developed methodology for assessing uncertainty can also be used to investigate improvements in uncertainty evaluation for other nanomaterial control banding tools.

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6.10.T-05 Prospective Material Flow Analysis of Advanced Materials: Building a European Circular Economy?

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Most studies analyzing mass flows of nanomaterials only covered recycling as a sink compartment and do not cover recycling flows because the advanced material under study is not intentionally targeted for this purpose. Thus, this study aims to explore potential flows of advanced materials in future scenarios, focusing on the end-of-life and recycling stages, using graphene in tires, batteries, and concrete to showcase possible consequences of the material circularity approach and exemplify connections in a European circular economy context. We used the dynamic and probabilistic material flow analysis model and three prospective scenarios to explore potential flows and evaluate potential dynamic changes in the system if Europe moves towards a circular economy. According to the model and scenarios, technical sinks and compartments out of the system would be significantly influenced, while environmental compartments are slightly influenced. Finally, using the combined system (tires-batteries-concrete), we observed that concrete might be a final sink for materials in the future that was not considered previously. Therefore, more research is needed to understand the implications of graphene (and other materials) during recycling streams and evaluate the implications of using recycling residues as backfillers in concrete due to the potential challenge of accumulating mixtures, which might have an impact on the recyclability of concrete in the future.

6.10.P From Nanomaterials to Advanced Materials: Challenges and Progress in Research, Industrial Application and Regulation

6.10.P-Th368 Bioaccumulation of Silver Nanoparticles (AgNPs) Induces Autophagic Dysfunction in Adult Intestinal Stem Cells to Accelerate Functional Aging

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Silver nanoparticles (AgNPs) are widely used in various consumer products due to their potent antibacterial properties. However, exposure to AgNPs presents significant biological and environmental risks. AgNPs induce acute toxicity through the disruption of autophagy. Moreover, our recent findings indicate that AgNPs cause long-term toxicity and impair intestinal function through lifetime bioaccumulation. However, it remains unclear whether, and how, AgNPs dysregulate autophagy in intestinal stem cells (ISCs) to accelerate aging. In this study, we used *Drosophila* as an *in vivo* model to investigate the role of autophagy in AgNP-induced adverse effects on longevity. We first demonstrated that early-life exposure to AgNPs leads to developmental lethality and reduces lifespan in a dose-dependent manner, consistent with our previous findings. Furthermore, AgNPs bioaccumulate in the adult intestine, resulting in the loss of intestinal integrity. Mechanistically, AgNPs cause autophagic dysfunction and subsequently reduce the ISC population in the adult posterior midgut as aging progresses. Interestingly, in the larval midgut, AgNPs induce both autophagy and apoptosis. Knockdown of autophagy-related genes in larval ISCs mitigates AgNP-induced toxicity during development, whereas overexpression of these genes in larval ISCs decreases survival following AgNP exposure. Taking together, our results suggest that AgNP-induced autophagic dysfunction in adult ISCs depletes the ISC pool, thereby accelerating aging and associated functional declines. This study provides the first evidence that autophagy in ISCs mediates AgNP-induced adverse effects at the molecular, cellular, and organismal levels.

6.10.P-Th369 Harmonisation of *Daphnia* sp. Standardised Protocols: Assessment of Nanomaterials' Behaviour

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The characterisation and behaviour assessment of nanomaterials (NMs) are crucial for harmonising Guidance Documents (GD) and Test Guidelines (TG) for ecotoxicity testing. These guidelines are essential in ensuring compliance with European regulations like REACH and improving risk assessment of NMs. The OECD GD No 317 outlines comprehensive recommendations for modifying existing OECD TGs for aquatic and sediment toxicological assessments of NMs, addressing the unique challenges of unstable NM and focusing on strategies to minimise agglomeration/aggregation and optimise dispersion stability during testing. This study aims to understand the behaviour of ZnO NMs, TiO₂ NMs, bentonite nanoclays and multi-walled carbon nanotubes (MWCNTs) (JRC supply) in stock dispersions and test medium (ISO) of *Daphnia magna* and the impact of various exposure methods in their stability, to adapt the OECD Acute Immobilisation Test (No. 202) for *Daphnia* sp. Activities involved 1) assessing the dispersion efficiency of the four NMs in stock dispersions, 2) evaluating the stability of the NMs in the ISO medium over time (48h) and utilising different experimental configurations to determine the influence of container material (plastic versus glass) and depth (plastic tube versus plastic petri dish) on NM behaviour. Results showed that stock dispersions of ZnO, TiO₂, and bentonite NMs exhibited reasonable stability in the stock dispersions (Milli-Q water), with TiO₂ NM demonstrating the highest stability over the 48h (30.23 ± 1.50 mV at 24h; 31.27 ± 11.87 mV at 48h), and bentonite the lowest. The NMs' zeta potential (ζ P) values fell within the range indicative of unstable colloidal dispersions (-30 mV to +30 mV) in ISO media. TiO₂ and bentonite NMs showed low variation in ζ P, with percentages of 2.86% (25.6 mg/L) and 4.55% (256 mg/L) for TiO₂, and -4.95% (25.6 mg/L) and -6.48% (256 mg/L) for bentonite. The hydrodynamic size of the particles was notably variable. TEM results indicate a more efficient dispersion of MWCNTs with NOM. These results will integrate the toxicity assessment results for *D. magna* exposed to these NMs under the same experimental setup. (ecotoxicity results presented at SETAC Seville). The combination of previous ecotoxicity results with this characterisation data provides important information and recommendations, which are being included in the OECD GD No 317 as annexes to aid in standardising techniques and methodologies for assessing NM hazards in *D. magna*.

6.10.P-Th370 Effects of Naproxen in Soluble vs. Nanostructured Form on the Zebrafish *Danio rerio*

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Inorganic nanoengineered drug delivery systems (NDDS) have been designed and used to release active ingredients in a controlled way, thereby ensuring the optimal drug concentration in the organism. Due to their widespread use, these NDDS may end up in wastewater. Since wastewater treatment plants (WWTP) often cannot adequately remove them, they can reach aquatic organisms and cause undesired effects. The ecotoxicological assessment of such novel pharmaceuticals is, thus, crucial to derive an accurate environmental risk assessment (ERA) by developing and/or adapting standardized testing procedures and improving risk-based regulations. Therefore, the present study aims to evaluate the effects of naproxen, a non-steroidal anti-inflammatory drug, in zebrafish (*Danio rerio*), in its sodium salt form as well as in a novel nanostructured form. The nanoform was achieved by naproxen intercalation in magnesium and aluminium-layered double hydroxides (LDH), a class of versatile stimuli-responsive anionic clays, used in a wide range of controlled release technologies.

The morphology, chemical composition, structural, textural, and thermal properties were investigated using a large suite of conventional techniques, namely TEM, SEM, ATR-FTIR, XRD, TGA, and N₂ adsorption isotherms. The loading content and release profiles were also determined using UV-Vis spectroscopy. For the ecotoxicological assessment, *D. rerio* embryos were exposed to aqueous solutions of naproxen sodium and dispersions of LDH-Naproxen, according to the OECD 236, and lethality and malformations were assessed after 96 h of exposure. In a sublethal test, biochemical markers of oxidative stress and neurotoxicity and behavioral endpoints were determined after 120 h of exposure.

Briefly, naproxen was successfully intercalated within the LDH galleries. Additionally, results showed distinct toxicity patterns of naproxen in both soluble and nanostructured forms to *D. rerio*. These findings will reinforce the environmental risk assessment (ERA) of naproxen and offer key data for the future ERA of the novel NDDS, promoting the development of sustainable and safe-by-design pharmaceuticals with reduced ecological impact.

6.10.P-Th371 Holistic Assessment of a Novel Antifouling Nanoadditive Based on a Natural Compound

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Marine biofouling is a natural process where fouling organisms adhere to immersed surfaces, causing extensive socio-economic and environmental impacts. Traditionally, antifouling biocides have been used in coatings to tackle this issue. However, these biocides pose environmental risks due to their persistence and toxicity. Consequently, there is a growing need for eco-friendly alternatives. Natural or Nature-inspired antifoulants (NIAFs) have emerged as a promising solution due to their lower environmental impact and sustainable synthesis. Researchers from the University of Porto have synthesized a natural compound exhibiting significant anti-macrofouling activity, low bioaccumulation potential, and preliminary low (eco)toxicity. Despite these advantages, a major challenge is expected: the premature release of this active agent from coatings due to its water solubility. Nanotechnology offers a potential solution to this problem. By immobilizing active ingredients in stimuli-responsive engineered nanomaterials (ENMs), it is possible to control their release over time, thereby extending the service life of the coatings and reducing their environmental impact. Thus, to develop long-lasting additives for coatings, this study aims to immobilize this natural antifouling agent into nanostructured materials, characterize the nanostructured forms, and assess their environmental behavior, ecotoxicity, and marine hazard compared to the soluble form.

Several nanoforms were developed with a loading content ranging from 22% to 40%. The toxicity of the soluble and most promising nanostructured forms ranged between non-toxic ($EC_{50} > 100$ mg/L; to the crustacean *Artemia salina*) to harmful ($10 > EC_{50} > 100$ mg/L) or toxic ($1 > EC_{50} > 10$ mg/L) to six species, while causing significant effects in embryo-larval stages of *Paracentrotus lividus* (at 1 ?g/L). Remarkably, the immobilization process reduced the NIAF toxicity on three tested species compared to the soluble NIAF. One of the developed nanoforms demonstrated its potential as a promising, eco-friendly, and long-lasting antifoulant solution, having the lowest release over time and lower ecotoxicity than the other nanostructured forms. This research represents progress towards sustainable antifouling solutions, demonstrating the potential of nanotechnology to address the limitations of current biocides. Finally, this study highlights the importance of a safer-by-design approach in developing eco-friendly antifouling technologies.

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6.10.P-Th372 An Assessment of the Toxicity of Nanogold Containing SARS Cov-2 Rapid Test Kits on *Daphnia pulex*

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The introduction and surge of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 virus) has resulted in the development and rise of various new fields of science and technology in efforts to combat it. Nanoscience and nanotechnology have emerged with materials that are essential in the fight against the Coronavirus (COVID-19). The rapid development and deployment of SARS-CoV-2 diagnostic test kits containing colloidal gold nanomaterials (nAu) has raised concerns regarding their environmental impact, particularly in aquatic ecosystems. This study evaluated the acute toxicity of the leached component in these rapid test kits on *Daphnia pulex*, a widely recognized bioindicator species which is sensitive to pollutants, using two exposure solutions a direct addition of test strips over exposure duration and a stock solution prepared from 138 test strips after 15 minutes of leaching. The solutions were used to expose *D. pulex* at varying leachate concentrations over 24 and 48 hours, monitoring immobilization as a primary toxicity indicator. Characterization of the nAu via Fourier Transform Infrared Spectroscopy (FTIR) and Dynamic Light Scattering (DLS) revealed an average particle size of 100 nm with a complex mixture of functional groups, including organic stabilizers, surfactants, and preservatives, that potentially influence their stability, reactivity, and toxicity in aquatic environments. Results indicated that nAu exposure led to significant *D. pulex* immobilization, with LC50 values decreasing over time, indicating heightened toxicity with prolonged exposure. Additionally, CytoViva dark-field hyperspectral microscopy confirmed the uptake and distribution of nAu in *D. pulex*, particularly in the digestive tract, indicating ingestion as a key exposure pathway. These findings highlight the potential risks posed by nAu-containing diagnostic kits to aquatic ecosystems. The study emphasises the need for careful assessment and regulation of nanomaterials in consumer products to prevent unintended environmental harm. Further research on long-term effects, bioaccumulation, and mitigation strategies is recommended to inform safer nanomaterial use and disposal practices.

6.10.P-Th373 Health and Environmental Issues Related To Advanced Materials: Implementation of Safe and Sustainable by Design Framework from SUNSHINE to SUNRISE Project

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The safety and sustainability performances of advanced materials (AdMa) should be critically assessed throughout their lifecycle, from development to end-of-life. As engineered nanomaterials (NMs), AdMa are intentionally produced to possess unique properties that enable innovation across various industries, such as electronics, healthcare, construction, and energy. However, like NMs, the innovative nature of AdMa implies that their risk assessment is still challenging. Thus, a valuable approach to guide the development of AdMa throughout the entire value chain, minimizing their potential risks to human health and the environment could be the European Commission's Safe and Sustainable by Design (SSbD) framework for chemicals and materials. The first three (out of five) SSbD steps address different safety aspects: I) hazard assessment based on intrinsic properties; II) occupational health and safety (including exposure) during the production and processing phases; III) exposure during the final application phase. Therefore, this approach, recently adapted to multi-component nanomaterials (MCNMs) within the H2020 SUNSHINE project, could allow us to obtain useful information for AdMa safety assessment at very early stages of the innovation process. Within the recently started SUNRISE project, first efforts to assess safety of AdMa selected as case studies in SUNRISE will be devoted to extrapolate as much as possible information already available in the literature on similar materials and/or their individual components. The three case-study AdMa are designed for different industrial applications, such as offshore installations, cosmetic products and agricultural sector. This work will therefore present the selection of tools and

procedures, possibly borrowed from SUNSHINE project and other recent/ongoing Horizon Europe projects, specifically designed for early-stage innovation, which will allow a preliminary assessment of AdMa safety and the identification of first recommendations for process redesign. These outcomes are expected to be integrated in the overarching Integrated Impact Assessment Framework (IIAF) to be developed in SUNRISE to support SSbD decision making for products enabled by AdMa.

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6.10.P-Th374 A Foresight Process To Identify Upcoming And Emerging Advanced Materials And Chemicals To Support Regulatory Preparedness

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The pace at which new advanced materials (AdMa) and Chemicals are being developed and brought to market continues to increase, with regulators often not being able to keep up with the pace of knowledge generation and innovation of AdMa. A Foresight Process has been developed in the Horizon 2020 SUNSHINE project (h2020sunshine.eu) as a tool to help identify upcoming and emerging AdMa and chemicals at an early stage in their development, enabling regulators to evaluate whether current regulation covers the emerging AdMa, and by extension chemicals, or if action is needed to update or modify existing regulations.

The Foresight Framework comprises two stages:

Using the TIM search engine developed by JRC (<https://www.timanalytics.eu>), text mining of selected literature sources such as SCOPUS, patent databases, Cordis and others is done utilizing selected search terms and strings. A targeted search brings up keywords (weak signals) which indicate possible emerging and innovative AdMa. The search can be repeated as often as needed using a more targeted set of selected search terms.

The second stage is refining and evaluation of these keywords or signals, done by a team of experts from the regulatory community, industry and others, working within a Trusted Environment, delivering a final short list of the signals corresponding to the most trending, upcoming and emerging AdMa.

A pilot study of the Foresight Process has now been completed as proof of concept, where the initial 20,000 results from the TIM search were reduced to a manageable number and refined by an expert team into five most promising signals of new and emerging AdMa.

The results of the Foresight Process can be fed into tools such as Early4AdMa, a pre-regulatory and anticipatory risk governance approach for AdMa helping regulators identify potential issues of an AdMa, related to their safety, sustainability and/or regulatory needs, at the early stages of their development or use.

The creation of standardised, harmonised search procedures established in the SUNSHINE Foresight process, including a dedicated Trusted Environment, will enable regulators to be informed at an early stage whether innovations are sufficiently covered by existing regulatory frameworks.

The development of the Foresight Process is being continued in the Horizon Europe SUNRISE project.

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6.10.P-Th376 Design, Tiered Assessment, Benchmarking and Re-design of Advanced Materials: Two HARMLESS Case Studies

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Advanced materials are novel by their very nature. The assessment of their positive and negative impacts on sustainability, environmental safety and human safety, is more uncertain than with conventional materials, and should start at the earliest phases of innovation. Here we utilize structured guidance for the design and assessment of advanced materials, supported by categorizations and comparative screening approaches, e.g. known sustainability issues of the sector of intended use or known safety issues of specific material classes. The used tools were tiered according to the StageGate innovation process. We report on two case studies of advanced materials that replace conventional materials in different industry

sectors and induce positive or negative impacts on different sustainable development goal (SDG) targets: Automotive exhaust catalyst with doped oxide-perovskites (La, Co, Ni, Pd, Pt):

- trade-offs between SDG targets 12.2 and 11.6.
- screening by prioritized NAMs: systematic trend with metal composition
- performance benefit by oxidative storage capacity, but the primary property of catalytic conversion is below benchmark.
- preferred SSbD candidate identified, but project likely to be stopped.
Façade insulation with aerogel-fibre.mats
- trade-offs between SDG targets 11.1 and 3.9. Ambiguous on SDG 12.2 (conflicting impacts during production vs during use phase).
- screening by prioritized NAMs: similarity of released dust to known silica.
- performance benefit over conventional material.
- preferred SSbD candidate identified, recommended for pilot with exposure management for the professionals installing the panels on façades.

We strongly differentiated the depth of consideration and testing between the ideation and business case phase and laboratory phase. We performed benchmarking of performance to the conventional material, and comparative assessment by NAMs for the prioritized safety concerns (or hazard and exposure). The used tools are available at <https://diamonds.tno.nl/projects/harmlesspublic>. The rationale to the first of three tools is already published (DOI: 10.1039/d3en00831b). None of the screening approaches targets the level of certainty of regulatory assessment, which remains reserved for the few materials that reach market introduction.

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6.10.P-Th377 Advanced Materials (nanoagrochemicals) in the Triple Planetary Crisis Perspective: Impact of Temperature Change and Pollution on Common Soil Invertebrates

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Agricultural practices are under pressure to deliver food in more sustainable methods, less polluting, and at the same time climate change create further challenges. Advanced materials (AdMa) like nanopesticides can provide safer and more environmentally sustainable alternatives to conventional pesticides, due to their increased effectiveness, reliance on less harmful substances, and more targeted delivery. There is an absence of environmental risk assessment regarding the impact of AdMa pollution and climate changes, in particular on important groups of soil-dwelling organisms.

Karate Zeon®, a commercial insecticide with reported nanofeatures was investigated, using the soil model invertebrate *Enchytraeus crypticus* (Oligochaeta) using LUFA 2.2 soil. This was compared to the active substance lambda-cyhalothrin. Temperature variations were tested beyond the standard 20°C: 16°C, 20°C and 26°C. Testing covered a long range of endpoints and life-stages that are beyond the standard endpoints, from short- to long(er)-term exposures, including: avoidance behaviour (2 days), OECD standard reproduction test (28 days): survival, reproduction, plus adults' size, and its extension (56 days): total number organisms; and Full Life Cycle (FLC): hatching and juveniles' size (13 days), survival, reproduction and adults' size (46 days). Toxicity of Karate Zeon® changed with the different temperatures. In terms of avoidance response, unlike at 20°C (for which no avoidance response was observed), enchytraeids tend to avoid the spiked soil at both 16 and 26°C, with highest avoidance at 26°C. Regarding survival and reproduction (OECD standard and its extension, and FLC test), there was an overall increase in toxicity with temperature drop from 20 to 16°C, and a decrease in toxicity with heat, from 20 to 26°C. Here we observed that temperature changes altered the toxicity of Karate Zeon®, as assessed based on the standard test conditions. Both the temperature increase and decrease affected the environmental toxicity of AdMa in unpredictable ways; hence there is an urgent need to narrow the current information gap concerning the interplay between the three interlinked issues constituting the Triple Planetary Crisis.

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6.10.P-Th378 Sustainable Nano Strategies for Water Remediation – Estarreja as a Case Study

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Seawater intrusion, eutrophication and metal contamination seriously impact coastal regions due to their location and higher industrialisation. Nanotechnology-based tools, such as layered double hydroxides (LDHs), may be remediation alternatives with higher eco-friendly potential than current options on the market. These anionic nanoclays have the capacity to exchange the stabiliser anion in their interlayers with anions in the surrounding media, such as chlorides or phosphates, constituting promise remediation solutions for waters at risk of salinisation or eutrophication. This work investigated the potential of LDHs as an environmentally safe alternative to remediate waters. Freshwaters from the Estarreja municipality (Aveiro, northern Portugal) were used as a case study due to the low-lying coastal lagoon location and historically contaminated waters from this region's intense industrial and agricultural practices. Activities involved 1) sampling water from several sites in Estarreja, 2) analysing anion and metal concentrations, 3) performing water remediation tests from three selected sites, 4) chemical analysis and ecotoxicity assessment of the remediated waters with four freshwater species: *Raphidocelis subcapitata* (microalgae; OECD 201), *Lemna minor* (macrophyte; OECD 221), *Daphnia magna* (OECD 202), *Danio rerio* (zebrafish; OECD 236). Magnesium-Aluminium LDHs stabilised by nitrates (MgAl-NO₃ LDHs) in slurry or calcined form were used. Remediated samples showed similar chloride levels from the non-treated ones, while phosphate, sulphate and metal (Zn, Al, As, Fe and Mn) concentrations were generally significantly lower in the treated waters than in non-treated. Overall, daphnids survival, zebrafish survival and hatching success and the growth rate of the microalgae and the macrophyte were significantly lower in exposures to non-treated samples than in those treated with LDHs. Results show the eco-friendly potential of these LDHs to remediate waters impacted by eutrophication and metal contamination. However, more studies are needed to better understand their efficacy in remediating waters affected by salinisation. These data are important to support LDHs as an eco-friendly alternative for water remediation and their industrial application. Furthermore, these results can aid in the regulation and risk assessment of these emerging nanoforms.

6.11.P Flame Retardants Regulatory and Circular Economy Challenges

6.11.P-Tu492 An Industry Perspective - How are the Producers of Phosphorus, Inorganic and Nitrogen Flame Retardants (Pinfa) Reacting to the Regulatory and Circular Economy Challenges?

Adrian Beard¹ and Francesca Filippini², (1) Clariant, Germany, (2) Cefic, Belgium

Flame retardants (FRs) have been under scientific and regulatory scrutiny since the 1990s, following the widespread detection of certain brominated FRs in the environment and the discovery of their problematic environmental and health effects. This led to regulatory restrictions on several substances under national and regional regulations. To replace these "legacy" FRs, new products were developed, often based on non-halogenated phosphorus, nitrogen, or inorganic chemistries. However, some of these alternatives, phosphate esters and nitrogen compounds, have also been found in the environment and living organisms.

In 2009, the industry group for phosphorus, inorganic and nitrogen flame retardants (pinfa.org) was established with the mission to develop and promote sustainable flame retardants. This presentation will discuss how pinfa has responded to the European Chemicals Strategy for Sustainability by creating a roadmap with specific actions. pinfa has engaged with the European Chemicals Agency (ECHA) on their Flame Retardants Regulatory Strategy, providing input on substance grouping of phosphorus-based FRs and discussing additional data needs. Besides generating new data, pinfa emphasizes the importance of communicating existing data and contextualizing analytical findings with risk evaluation. pinfa member companies have conducted case studies on Safe and Sustainable-by-Design FRs, with some support from the Joint Research Centre (JRC). One key lesson learned is that this new approach requires practical implementation tools.

The circular economy presents challenges for plastics in general, as the wide variety of additives, fillers, and pigments complicates recycling. However, simply eliminating additives (including FRs) is not a

viable solution, as they serve important purposes. pinfa has undertaken projects on mechanical recycling involving FRs and advocates for increased collaboration along the value chain and improved design for recycling to address these issues effectively.

pinfa members believe that FRs contribute to making products safer for everyone, particularly in terms of fire safety, and that their risks are very low making them safe for both the environment and humans. Nevertheless, they acknowledge the need to proactively address concerns and support appropriate regulation of FRs with critical findings.

6.11.P-Tu493 Safe and Sustainable by Design, Scoping, and Simplified Assessment of 30 Alternative Flame Retardants for Use in Polymer Insulation Foams

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The European Green Deal underscores the need for a Safe and Sustainable by Design (SSbD) strategy for chemicals and innovative materials. This initiative, along with the Ecodesign for Sustainable Products Regulation, marks a significant step toward promoting environmentally friendly practices in product design and manufacturing, facilitating the transition to a more sustainable economy in Europe. To implement this strategy effectively, the Joint Research Centre (JRC), the World Business Council for Sustainable Development (WBCSD), and the European Chemical Industry Council have developed guidelines outlining criteria for SSbD and providing data-driven recommendations. However, few cases have successfully implemented SSbD in research and development projects.

The PLANETS project addresses this gap by promoting the development and implementation of straightforward, effective, and cost-efficient SSbD strategies for materials and products through a tiered approach. This begins with scoping and simplified assessments in Tier 1, followed by risk screenings combined with life-cycle assessments (LCA) using existing data in Tier 2, and culminating in experimental methods alongside comprehensive LCA in Tier 3. In this work, we focused on applying Tier 1 assessments to identify alternatives to brominated polymeric flame retardants. Halogenated flame retardants containing bromine or chlorine release free radicals that disrupt combustion but face regulatory scrutiny. Phosphorus-based flame retardants promote char formation while producing non-flammable gases. Nitrogen-based flame retardants release nitrogen gas during decomposition, helping dilute flammable gases, while inorganic flame retardants, such as aluminum hydroxide, release water vapor when heated, providing cooling effects.

We assessed over 30 SSbD alternatives to brominated flame retardants, including polyFR and HBCD, comparing alternatives such as s-triazine phosphonate, ammonium polyphosphate, organic phosphate, DOPO-P-S polymer, and styrene-organophosphorus-sulfur copolymer. The target use is in insulation foams made from polyurethane, polyethylene, and polystyrene. Alternatives were evaluated based on hazard profiles and categorized by performance, compatibility, cost, degradation products, and recyclability. By utilizing the ECOTOX and ECHA databases, along with Safety Data Sheets and the HARMLESS Decision Support System tool, we identified the most suitable alternative flame retardants.

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6.11.P-Tu494 Quantifying Efficiency of Managing POPs Waste in the UK

Richard McKinlay, Water Research Centre Ltd, United Kingdom

Substances such as brominated flame retardants (BFRs), which are now restricted in use and classified as POPs, were previously added to a range of products at the point of manufacture. These legacy products are now being disposed of alongside products made after restrictions were introduced, so it is vital to determine which waste streams should be classified as POPs, to ensure they are correctly managed.

WRc has carried out several studies with support and funding from the Department for Environment, Food, and Rural Affairs (Defra), the Environment Agency and industry trade associations. Sampling and analysis of a variety of waste electrical and electronic equipment (WEEE) and waste domestic seating was conducted to quantify polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD). The separation efficiency of WEEE recyclers to concentrate POPs into a heavy plastic fraction to minimise the quantity of POPs waste requiring destruction was studied. The destruction efficiency of energy from waste (EfW) incineration processes, the primary disposal route for POPs waste was also

calculated.

It was concluded that a number of categories of WEEE, including display equipment and small mixed WEEE (SMW) contained items with PBDEs over 1000 mg/kg and therefore should be classified as POPs waste. Similarly a number of soft furnishings samples showed elevated PBDE and HBCDD levels in textile and faux leather coverings, leading to their classification as a POPs waste.

A mass balance study demonstrated that WEEE plastic recyclers could concentrate 85-95% of POPs into a dense plastic fraction, allowing for the production of a light fraction with <100 mg/kg PBDEs to be recycled. Using a mass balance approach, it was calculated that EfWs could be used to destroy PBDEs in WEEE waste with a destruction efficiency of 99.927% to 99.952%.

The combination of studies demonstrates how the UK has identified and classified two waste streams as POPs, as well as demonstrating the efficiency of the downstream separation and ultimate destruction in existing infrastructure. Analytical challenges remain in quantifying POPs in waste, and with the expansion of substances classified as POPs and the requirement to evaluate more waste streams this will remain a highly active area of research

6.11.P-Tu495 The Emergence of Alternatives to Restricted Brominated Flame Retardants in the Irish and UK Waste Streams

Stuart Harrad Prof¹, Martin Sharkey², William A. Stubbings Dr¹, Daniel Drage Dr¹ and Marie Coggins²,
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The presence of restricted brominated flame retardants (BFRs) such as hexabromocyclododecane (HBCDD) and polybrominated diphenyl ethers (PBDEs) is well-established in building insulation foam, waste electrical and electronic equipment (WEEE), end-of-life vehicle (ELV) waste, and waste furniture fabrics and foams. Given these restrictions, this paper tests the hypothesis that alternative flame retardants are increasingly to be found in such waste streams. In mixed engineering plastic from a UK WEEE recycling plant, maximum concentrations of decabromodiphenyl ethane (DBDPE) and 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE) were 4,200 and 27,000 mg kg⁻¹ respectively. By comparison, while trace concentrations (<1.1 mg kg⁻¹) of pentabromobenzene (PBBz) and hexabromobenzene (HBBz) were observed, no other alternative FRs were detected. In UK waste furniture fabric covers and foams, DBDPE was detected at concentrations up to 11,200 mg kg⁻¹. In samples of WEEE, ELV, building insulation, and soft furnishing waste from Ireland, anti-dechlorane plus and DBDPE were the only alternative FRs detected in at least one waste category at a detection frequency >20%, at maximum concentrations of 96 and 1100 mg kg⁻¹ respectively. However, 2,4,6-tris(2,4,6-tribromophenoxy)-1,3,5-triazine (TTBP-TAZ) was detected in 3 WEEE samples at between 14,000 and 32,000 mg kg⁻¹. Moreover, a TV contained 1,100 mg/kg DBDPE, while maximum concentrations of tris(1-chloro-2-propyl) phosphate (TCIPP), and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) in ELV foams, furniture and mattress foams, and building insulation foams were 100,000 and 340,000 mg kg⁻¹ respectively. Finally, in foams and fabrics from waste childcare articles (e.g. car seats) collected in Ireland, there were very substantial levels of TCIPP and TDCIPP (up to 170,000 and 390,000 mg kg⁻¹) as well as of: 2-ethylhexyl tetrabromobenzoate (EH-TBB) (100,000 mg kg⁻¹), DBDPE (9,600 mg kg⁻¹), and bis(2-ethylhexyl)tetrabromophthalate (BEH-TEBP) (39,000 mg kg⁻¹). Overall, the evidence suggests a very discernible presence of HBCDD and PBDE alternatives in waste polymeric goods in Ireland and the UK. Aside of the potential environmental impacts of such alternative FRs, their presence impedes the use of screening tools such as XRF that measure Br as an indicator of whether waste complies with regulatory limits on restricted BFRs. Further studies are required to establish whether this presence is increasing over time.

6.11.P-Tu496 PBDEs in Recycled Fertilizers – Implications for the Circular Economy

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A baseline assessment of PBDEs was performed in biosolids emanating from a selection of wastewater treatment plants (WWTPs) in Ireland. 21 biosolid samples, intended for use as agricultural fertilizer, were collected from 7 WWTPs over 4-month intervals in January, May, and September 2023. Highest concentrations of BDE-209 were detected in biosolid samples penta-/octa-BDEs were comparatively low. In biosolid samples, levels of BDE-209 were on the higher end of figures reported worldwide. As legacy PBDEs have been listed as persistent organic pollutants (POPs) though no legislation currently requires monitoring for their presence in wastewater, an investigation of potential contamination of the food chain through land-spreading of biosolids on agricultural lands would be warranted. PBDEs have a propensity

for partitioning into the solid fraction of waste, though it is unclear how these chemicals partition into different fractions/matrices after the application of biosolids to land. In the transition to a circular economy, the reuse of wastewater and resulting biosolids as nutrient-rich fertilizer is highly meritorious. Further research is therefore needed to determine the environmental fate of these chemicals following the use of biosolids on agricultural lands. The relevant regulations and guidance on reuse of WWTP-derived biosolids should furthermore be updated to consider a broader suite of contaminants such as PBDEs.

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6.11.P-Tu497 Questionable Safety vs. Known Risks: Reevaluating Flame Retardants in Lithium-Ion Battery Enclosures Amid New Safety Standards and Regulations

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Lithium-ion batteries are increasingly being adopted in a range of products, with forecasts suggesting that their market value will surpass \$400 billion by 2030. Although lithium-ion batteries contribute to net-zero emission goals, their use can cause dangerous fires. In light of this, new safety standards and regulations are emerging that could result in the unnecessary use of flame retardants without proven fire-safety advantages. In contrast, human and environmental harms from flame retardant use are well-established. Standards do not directly mandate flame retardants; however, adding them to plastics remains a simple and cost-effective method to comply with open-flame testing requirements. Plastics that meet UL94 V-0 ratings often contain 10-20% flame retardants by weight. Major uncertainties prevail regarding the actual fire-safety efficacy of these flame-retarded enclosures, including whether they effectively stop thermal runaway or potentially contribute to increased gas and particle emissions. Publicly available data does not substantiate the fire-safety benefits, while the hazards of flame retardants are well-documented. Many flame retardants present risks throughout the lifecycle of lithium-ion batteries, from production to disposal. Occupational exposure during manufacturing and contamination of nearby communities pose severe health and environmental threats. Flame retardants migrate from plastics over time, accumulating in buildings and ecosystems. Fires involving such chemicals yield toxic byproducts such as dioxins and furans, increasing the incidence of fire fatalities and injuries due to smoke. Furthermore, end-of-life challenges, such as impaired recycling, are exacerbated by these chemicals, and can lead to continuous exposure.

This poster will highlight concerns about the use of flame retardants in lithium-ion battery enclosures, drawing from evidence that such chemicals, while intended to meet flammability standards, may cause significant health issues without clear safety benefits. We will also present potential alternatives to the use of flame retardants. Ultimately, we aim to highlight that scientific evaluation is needed to assess the safest and most effective ways to meet fire safety standards and regulations, given the increasing use of rechargeable batteries.

6.11.P-Tu498 Legacy Flame Retardants: A Key Challenge for the Transition to a Circular Economy for Plastics in Building and Infrastructure

Sarah Schmidt¹, Thomas Gibon² and David Laner¹, (1)University of Kassel, Germany, (2)SUSTAIN research unit, Luxembourg Institute of Science and Technology (LIST), Luxembourg

Recycling schemes for plastics in long-lived products are challenged by the presence of legacy contaminants that have been phased out in virgin materials and are restricted in new products. An example is the brominated flame retardant hexabromocyclododecane (HBCD), which was used in insulation materials made of expanded polystyrene (EPS) and extruded polystyrene (XPS).

As part of the research project IMMEC, funded by the Luxembourg National Research Fund (FNR) and the German Research Foundation (DFG), trade-offs between the phase-out of legacy contaminants and the establishment of a circular economy are assessed for plastics in buildings and infrastructure. A high-resolution dynamic material flow model has been developed to quantify historical and potential future stocks and flows of plastics in the building and infrastructure sector in Germany, accounting for legacy contaminants. The dynamic material flow model is coupled with prospective life cycle assessment to evaluate different management strategies from a contaminant as well as from a life cycle impact perspective. HBCD in EPS and XPS insulation serves as a case example within this model.

The dynamic material flow analysis shows that, in 2023, Germany's stock of insulation materials made of EPS and XPS reached approximately 9.3 million tons, with 70% contaminated by HBCD. Although HBCD was phased out of EPS and XPS production in 2015, and recycling of HBCD-contaminated

plastics is avoided to prevent reintroducing legacy contaminants, the model indicates it will take another 35 years from now for contaminant-free waste volumes to surpass contaminated waste. While this poses a challenge to circular material management strategies, refined waste management concepts may offer pathways to phase out HBCD and simultaneously enable enhanced material recovery resulting in lower life cycle impacts.

Particularly for long-lived products like plastics in the building and infrastructure sector, the phase-out of legacy contaminants challenges the transition towards more circular material systems. As demonstrated for HBCD in insulation materials, legacy additives may contaminate waste flows for many decades. Although restrictions can accelerate the phase-out of harmful substances, they counteract efforts to close material cycles and reduce climate impacts. Therefore, comprehensive analyses are needed to identify environmentally optimal waste management approaches and to support robust decision-making.

Track 7. Moving Beyond - Cross Cutting Themes, Emerging and Transdisciplinary Topics

7.01.P Framework for Post-Conflict Environmental Damage Assessment

7.01.P-Mo474 Applying the US Natural Resource Damage Assessment Framework to Post-Conflict Damage Assessment

Richard J Wenning¹ and Ted Tomasi², (1)Wenning Environmental LLC, (2)Integral, Inc., USA

Military conflicts in Ukraine and other regions worldwide highlight the social, economic, and environmental impacts on local communities, raising the question of how to quantify the damage to natural resources caused by armed conflict and implement restoration efforts. Applying a Natural Resource Damage Assessment (NRDA), similar to the method used in the US to evaluate industrial accidents, can enhance procedures implemented elsewhere. Several notable examples exist where similar principles and frameworks have been applied in arbitration and law courts to resolve international disputes, including determining post-conflict reparations. Various governments and international organizations have utilized NRDA-like approaches to assess damage to natural resources, estimate the costs of repairing, restoring, or replacing the damages, and prioritize recovery efforts. A well-informed damage and recovery assessment framework should (i) address the needs of the affected people and regions, (ii) foresee potential outcomes different from pre-conflict conditions that reflect new or evolving social needs, and (iii) guide the prioritization of reconstruction efforts based on environmental risks. The latter is especially crucial as financial and public resources may be limited. This presentation outlines an NRDA-type framework applicable to environmental assessment and recovery following armed conflict. This framework stresses the necessity of being fact-based, scientifically sound, and transparent to the extent possible. It emphasizes collaboration with international recovery efforts to rebuild critical infrastructure and restore essential environmental services (such as land safe for farming and accessible potable water) and social well-being. The goal is to provide a method to optimize available financial resources, accelerate resource recovery, and restore local communities stability and safety.

7.01.P-Mo475 Assessment of Environmental Impacts Due to the War in Ukraine

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Since the Russian invasion of Ukraine in April 2022, concerns have been raised not only about the severe humanitarian impact but also about the environmental damage caused by harm to Ukraine's biodiversity and infrastructure. Particularly regarding biodiversity, international efforts to enhance conservation have been promoted since the adoption of the Kunming-Montreal Global Biodiversity Framework as a global biodiversity target at the 15th Conference of the Parties to the Convention on Biological Diversity (COP15) held in December 2022. There are concerns that achieving the "30 by 30 target" established in this framework may be delayed due to the war.

This study focuses on the natural wildfires and infrastructure damage caused by the war to quantitatively assess its environmental impact. First, geospatial data analysis was used to identify the extent of the fires and infrastructure damage, as well as the amount of stock loss in building materials. Subsequently, Life Cycle Impact Assessment (LCIA) methodologies were employed to quantify the environmental impacts resulting from these factors.

In the regional assessment, it was found that, despite the northern and eastern regions of Ukraine being dominated by farmland, wildfires had a significant impact on grasslands and forests. This is believed to be

due to ground battles being concentrated in areas consisting of grasslands and forests, avoiding the open farmland. Additionally, the high density of vegetation in forests and grasslands likely facilitated the spread of fires.

In contrast, the southern region experienced the most significant wildfire damage in wetlands. This is thought to be the result of frequent attacks on port facilities and grain storage facilities along the Black Sea. Many wetlands in the southern region are designated as ecological reserves, raising concerns about the greater impact on these areas compared to other natural environments.

In assessing infrastructure damage, it was shown that a substantial amount of stock was lost. The reconstruction of these damaged infrastructures will require the production and transportation of additional construction materials, raising concerns about the environmental impacts associated with recovery efforts.

7.01.P-Mo476 Environmental Resilience during Prolonged Armed Conflict

Igor Linkov¹ and Tatiana Biloborodova², (1)US Army Corps of Engineers, United States, (2)Academia, Ukraine

The war in Ukraine has caused unprecedented damage to civilian infrastructure over extended periods. Russian artillery, drone, and missile strikes on Ukraine's energy generation and transmission systems have significantly reduced the available power supply. These prolonged attacks pose new challenges for infrastructure and environmental recovery. Developing prioritization methods for recovery is critical to building resilient systems under the pressures faced in prolonged peer and near-peer conflicts. In this presentation, we discuss two projects aimed at improving resilience to prolonged attacks. First, we describe an examination of the patterns and impacts of Russian attacks on the Ukrainian energy grid and determining which aspects of the energy infrastructure are most critical to restore in order to stabilize the overall system. Second, we explore the use of machine learning methods from drone and satellite data to prioritize environmental remediation interventions in fire-damaged areas. Specifically, this methodology attempts to use drone data to identify areas which are least likely to environmentally recover without human intervention, especially in environments where data collection would be operationally challenging. Overall, this work introduces new methods to understand the implications of a wide body of prolonged extreme events, including warfare and recurring climate extreme events such as wildfires, hurricanes, and floods.

7.02 Next Generation of Environmental Risk Assessment - From Data to Design

7.02.T-01 Development of a New Framework Advancing the Integration of New Approach Methodologies in Environmental Risk Assessment

Bruno Campos, Claudia Rivetti, Geoff Hodges, Iris Muller, Predrag Kukic and Jade Houghton, Unilever, United Kingdom

Conventional regulatory frameworks for assessing the environmental safety of chemicals are centred around the evaluation of in vivo data for standard ecologically representative species. This traditional approach is mostly based on apical effects studies performed on whole organisms. However, currently we witness a regulatory and ethical drive towards non animal approaches, incorporating New Approach Methodologies embedding greater mechanistic understanding of toxicological responses across species. Mechanistic-based approaches allow the generation of points of departure alongside the identification of the mechanisms of action across a variety of biological responses. However, there are still few examples of their implementation in Environmental Risk Assessment.

We present and discuss a conceptual framework for conducting safety assessments using a wealth of mechanistic data sources to inform safety and support the applicability of NAMS in ERA without the need for generating new animal data. We then validate the approach with a benchmarking exercise, exemplified with three case studies, including 17 β -Ethinyl Estradiol, Chlorpyrifos, and Tebufenozide. The overall strategy involves the collection and integration of all available effect data across human health and the environment (including historical in vivo, in vitro, and in silico endpoints) to demonstrate the suitability of mechanistic-based information to support and strengthen current practices. The results show this process represent a valuable step forward for robust environmental safety without additional animal testing, emphasizing the need for scientific consensus and further demonstration of NAMs' biological relevance and applicability with safety decision making processes.

7.02.T-02 Leveraging Cell Painting for Cytotoxicity and Mode-Of-Action Analysis of 1085 Diverse Compounds

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There is evidence that molecular profiling techniques, applied to cell models of varying complexity, may effectively detect adverse outcomes at the cell, tissue, or even organism level. Cell Painting is an image-based profiling assay using six fluorescent dyes to measure thousands of features for eight different cell components, including intensity, shape, texture, etc. In this study, we evaluate the potential for Cell Painting to provide cytotoxicity and mode-of-action information on pharmaceutical, agrochemical, and industrial compound exposures by predicting diverse in vitro assay readouts using Cell Painting data from primary human hepatocytes exposed to 1,085 compounds (8 concentrations ranging from 0.01 to 100 μ M). The in vitro assays included MTT and LDH assays that we measured, as well as publicly available ToxCast assays. We additionally investigate how different cell representations (traditional and deep learning-based) and exposure concentrations impact predictive performance. We found that Cell Painting was more sensitive than cytotoxicity assays - as judged by the number of active compounds and the relative activity concentration - suggesting that it captures rich mode-of-action signals. On average, image-based PODs were 2.5-fold lower than MTT, 7.9-fold lower than cell counts, and 15.8-fold lower than LDH. The choice of cell representation and profile summary method impacted image-based PODs, with DINO and CellProfiler able to detect activity for more compounds and at lower concentrations than CP-CNN. We evaluated the ability of Cell Painting profiles to predict the paired MTT and LDH assay readouts and found that models based on Cell Painting greatly outperformed simple models based on cell count, and that performance was similar across cell representations. For the ToxCast assays, we found that the predictive performance depended on experimental factors, with cell-based assays easier to predict than cell-free assays and liver-based assays easier to predict than assays derived from other tissues. Finally, we used the trained ToxCast assay models to predict molecular targets and modes-of-action for data-poor compounds that were not screened by ToxCast. This work will inform the future analysis of a large, multi-omics dose-response dataset generated by the OASIS Consortium consisting of Cell Painting, transcriptomics, and targeted protein data for ~1500 compounds in multiple biological contexts. This abstract does not reflect US EPA policy.

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7.02.T-03 Combination and Interpretation of Datasets with Mechanistic Models for Informed Decision Making and Increased Certainty in Regulatory Risk Assessment

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The environmental risk assessment of pesticides relies on endpoints for exposure and hazard which are used to receive risk quotients. For the hazard assessment the dataset submitted by the applicant is evaluated by the risk assessor study by study, species by species and as a result a single endpoint from one study is used to derive the risk assessment. Studies which are not meeting the reliability and relevance criteria are excluded from the analysis, which often occurs in the case of older studies. Therefore, valuable information about variability and uncertainty in the datasets for compounds is neglected or even lost. Consequently, the current regulatory system ignores a significant amount of available knowledge and information. A different approach is needed shifting to next generation environmental risk assessment, which will be accompanied by complex sets of non-animal data needing to be integrated into the regulatory system.

With this presentation we propose a different approach for the evaluation of large datasets using mechanistic effect models. With this approach, the complete dataset is used to inform the model which provides then an indication of the consistency of the data. Therefore, the model is able to investigate the full data package. If this analysis reveals a consistent pattern and the dataset is large enough, there is no need for the regulator to deep dive into the details of all underlying studies. If some of the data were incorrect or not consistent, the model would identify those as they would not fit in the overall picture. In case an inconsistency is discovered, more investigations are needed.

We will present examples of how we used this kind of approach in the registration process for our actives. Examples will cover aquatic invertebrates and bees, different models (GUTS, BeeGUTS and DEB) as well as different active ingredients.

Our study underlines the power of mechanistic modelling by integrating datasets which differ in exposure, biological level, or species. Following this approach opens the regulatory system for the next generation

risk assessment. Through the use of additional knowledge and mechanistic understanding, this approach allows to make a more robust and certain risk assessment and to integrate standard and non-standard test systems. In addition, following this approach potentially reduces resources to evaluate large datasets and makes risk assessment less complex.

7.02.T-04 Spot the Differences: Does Biotransformation Explain Sensitivity Variations in Non-Target Aquatic Arthropods to a Plant Protection Product?

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A major challenge in ecotoxicology is understanding why certain species are more sensitive to a given chemical than others. Typically, the effects of plant protection products (PPPs) on non-target aquatic arthropods are assessed through summary statistical metrics (e.g., NOEC, LCx). These are used either in isolation per species or combined in sensitivity distributions to inform regulatory decisions in environmental risk assessment. The impact of a chemical on different species can vary considerably and carbamates, for instance, can produce effects across aquatic invertebrate classes varying by up to four orders of magnitude between the most and least sensitive tested species. Attempts to explain and predict such sensitivity differences have traditionally relied on trait-based approaches that use morphological and ecological characteristics, while physiologically-related differences (such as biotransformation potential) have been poorly explored. Physiological traits, including a species' ability to modify or detoxify a chemical, have been shown to play a significant role in explaining toxicokinetic-related differences in sensitivity. In this study, we present the results of the exposure of six aquatic arthropod species (*G. pulex*, *L. benedenii*, *H. azteca*, *D. magna*, *C. riparius*, *C. dipterum*) to a carbamate PPP and identify the most and least sensitive species, in order to have a distribution of sensitivity. We then analyze the lethal effects with a reduced GUTS model (from the General Unified Threshold models of Survival framework) and doing so compare time-independent toxicity. In the next step, we aim to elucidate the mechanisms underlying the GUTS parameters obtained for two test species of interest. In a short-term exposure to carbaryl, we will assess following endpoints: i) uptake and elimination of the parent compound, measured through internal and external concentrations; ii) identification and internal concentrations of primary carbaryl metabolites; iii) biotransformation rates determined through in vitro and/or in vivo enzymatic assays. This methodological framework can be applied further to other species and eventually aid cross-species extrapolation, including predict toxicity in untested species. Identifying the key mechanistic determinants of toxicity, such as key elements of an adverse outcome pathway, aids cross-species extrapolation and ultimately provides a useful tool for development and regulation of safer PPPs.

7.02.T-05 Joining Structural and Genetic Similarity Modelling: Advancing QSAR for Cross-Species Toxicity Predictions

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In silico models are a valuable resource to current ecotoxicology because of the growing interest in the principles of the 3Rs for environmental risk assessment (replacement, reduction, refinement). Within nature, a vast number of species can potentially be affected by pesticide emissions. Consequently, there is a strong and growing societal need for assessing the effects of existing and novel pesticides.

Quantitative Structure-Activity Relationship (QSAR) models use chemical features to predict the toxicity of a compound based on its structural information. These models generally make use of the features extracted from the chemical structure and some biologically relevant features.

Here we present a QSAR model that also includes a species similarity metric so that the model can output fine-tuned predicted toxicity endpoints for a given species-chemical interaction. We incorporated genetic similarity as a proxy for common physiological responses and integrated chemical and genetic features into a QSAR feedforward neural network model.

We limited our predictions to LC50 data for a wide range of species and pesticides. The feature set of this QSAR model includes chemical structural features, exposure time, and species similarity. We thoroughly validated our model with repeated cross-validation over 100 independent iterations. We tested the prediction inside our defined applicability domain (interpolation). Our model demonstrates an average R² of 0.71 ± 0.10 and an RMSE of 0.82 ± 0.15 when predicting LC50 endpoints for species and chemicals in the applicability domain (interpolation). We also evaluated its potential to extrapolate an LC50 value for species outside the initial species set, or chemicals outside the initial chemical set, or for species and chemicals simultaneously outside the respective training sets.

7.02.P Next Generation of Environmental Risk Assessment - From Data to Design

7.02.P-We525 How FAIR is Ecotoxicology in the Era of Open Science?

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Environmental risk assessment of tomorrow requires a reliable and accessible knowledge base today. Despite the growing recognition of this need, studies indicate that most experiments are not reproducible, partially not even by the authors of the original studies. Factors that contribute to this circumstance are plenty and go beyond experimental aspects (e.g., low replication, imprecise methods, and random variation) toward the domain of data analysis. Availability of raw data and code for data analysis are essential for transparency allowing for the identification of potential errors, evaluation of uncertainties, and integration of past with present and future scientific outcomes. In this light, the FAIR guiding principles (i.e., findable, accessible, interoperable, and reusable) were postulated in 2016. These principles aim to stimulate data availability and transparency in science. However, scientific assessments of the status quo of data and code sharing are scarce and to best of our knowledge not existing in the field of ecotoxicology. To assess (1) how frequently raw data and code of original research publications are available in ecotoxicological studies and (2) whether the FAIR principles caused a paradigm shift over the last years, we conduct a comprehensive literature review focusing on environmental studies over a period of 20 years. Since the number of publications in this time frame is in the millions, we analyze a random and representative subsample (i.e., >2000 publications). Furthermore, in case raw data and code are available, we aim to rerun the analyses to evaluate reproducibility. Our contribution will not only assess the current situation in ecotoxicology but also animate peers to share their data and code in a reproducible way by informing about the methods (e.g., version management and control) and scientific incentives to do so.

7.02.P-We526 Digital Patterns for the Detection and Prediction of Chemically Induced Endocrine Disruption (ED) in Zebrafish Embryos (DiMEP)

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Endocrine-disrupting chemicals pose a significant threat to human and environmental health, by interacting with key physiological processes such as development and reproduction. However, the current evaluation of endocrine disruption is time-consuming, costly, and heavily reliant on animal-intensive in vivo studies. However, current evaluation of endocrine impairments requires the use of large amounts of animals through experimental testing. Zebrafish embryos present a promising model for reducing traditional in vivo vertebrate studies, as they are usually not protected by the EU animal welfare regulations until they first feed independently. Recent research has demonstrated that biomarkers of endocrine activity for some modes of action can be reliably detected in fish embryos.

In this project, we build upon these initial findings to investigate whether zebrafish embryos can be used to reliably detect endocrine activity across a range of known endocrine disrupting compounds that encompass a broad spectrum of endocrine modalities. The zebrafish embryos will be exposed to endocrine-disruptive chemicals with known estrogenic, androgenic, and steroidal modalities at sub-lethal concentrations until 96 hours post-fertilization and their toxicogenomic responses (i.e., RNA-seq fingerprints) will be assessed. Our analysis will focus on identifying expression signatures that are robust and specific to endocrine activity for the investigated modalities.

If such reliable signatures exist, toxicogenomic information could provide mechanistically informed insights into the endocrine activity of the tested chemicals, optimizing the testing strategy for endocrine disruption and reducing the need for unnecessary in vivo testing in accordance with the 3R principles.

7.02.P-We527 From Regulatory Decisions to Environmental Protection. Scientific Challenges for Informative Environmental Risk-Impact Paradigms

Jose V. Tarazona, Ana Fernandez Agudo, Noelia Dominguez Morueco and Maria del Carmen Gonzalez Caballero, Spanish National Environmental Health Centre. Instituto de Salud Carlos III, Spain

Our methodological capacity for conducting science-based environmental risk assessments (ERA) has significantly evolved in recent decades. In parallel, jurisdictions such as the European Union have strengthened the legislation, and environmental assessments are nowadays required for most chemicals. Nevertheless, these developments have not reverted the trend of environmental deterioration and biodiversity losses. This deterioration trend is the result of different factors including habitat loss, landscape intervention, climate change and other human interventions. Chemical pollution is identified among these factors, but due to the current ERA paradigm and despite the scientific developments we cannot quantify the actual contribution of industrial chemicals, pesticides, biocides, pharmaceuticals,

personal care products, etc. to environmental deterioration.

Our analysis suggests that the main limitation is that the current paradigm focuses on the short-term regulatory decision, e.g. authorization or use restriction, but does not allow to identify the residual risks and to integrate the risk characterizations into expected environmental impacts and their consequences. Based on this analysis, we propose the following key elements to be considered for developing a 21st century paradigm for ERA, focused on the identification of risk drivers, integration of environmental impacts at landscape levels, and the use of system-based approaches for moving from the quantification of risk to the identification of main environmental impacts and the expected short- and long-term consequences.

Key elements of the derivation of a new paradigm are the focus on New Approach Methodologies (NAMs), the consideration that in most cases the emissions and environmental effects are linked to human modified ecosystems, such as agricultural bio-systems and managed water bodies, and the integration of the environmental exposure of human subpopulations in the identification of environmental risk drivers.

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7.02.P-We528 FXMATE: Statistical Evaluation of Ecotoxicity Effects Made Totally Easy

Eric Bollinger, Dominic Englert, Felix Koenig and Jochen Zubrod, *Palinera GmbH, Germany*

Chemical registration requires numerous toxicity tests, creating a significant demand for time and specialized expertise across involved institutions. Despite the guidance provided, for instance by OECD, flawed statistical analyses remain a persistent challenge, potentially compromising the accuracy of toxicity estimates and jeopardizing the protectiveness of environmental risk assessments. Current practices often rely on custom code or proprietary software, which may lack transparency and introduce errors. FXMATE is designed to address these issues as an open-source tool that offers a structured approach to guiding users toward accurate, tailored analyses by making the latest R functionality available via a dockerized interactive Shiny app. Unlike a one-size-fits-all solution, FXMATE incorporates high-quality data validation, state-of-the-art dose-response modeling, and fail-safe hypothesis testing for NOEC/LOEC estimation. By automating guideline-specific data preprocessing steps (e.g., for OECD guidelines 201 and 211) and providing a user-friendly interface, FXMATE significantly improves both the quality and efficiency of statistical analyses. Beyond these core functions, the modular structure of FXMATE ensures extendibility for evolving risk assessment needs, while already providing unique features like bootstrapping, model averaging, and containerization to enable high reproducibility. FXMATE hence updates statistical analysis of ecotoxicity data to current methodological and software standards and supports academia, regulatory agencies, and industry in guaranteeing reliable future risk assessments.

7.02.P-We529 cleanventory: Harmonized Global Chemical Inventory Data for Assessing Persistence and Mobility Hazards

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Understanding the chemical diversity regulated across global trade markets is essential for informed decision-making and advancing regulatory frameworks. However, challenges such as limited public access to official documentation and diverse data formats hinder progress. With the growing number of regulated chemicals, a harmonized system aligned with the "one substance one assessment" principle is urgently needed.

Under the newly adopted CLP hazard classes for PMT and vPvM, persistence and mobility are central to chemical risk assessment. Persistence under CLP refers to a chemical's resistance to biodegradation, while mobility describes its potential to spread in the environment, particularly to water sources like groundwater and drinking water.

The H2020 ZeroPM project addresses these challenges through the development of the "cleanventory," an open-source global chemical inventory. Following FAIR principles (Findable, Accessible, Interoperable, Reproducible), the cleanventory features a user-friendly interface (<https://database.zeropm.eu>), regular updates on Zenodo, and open-source code on GitHub. This database integrates harmonized data from literature, QSAR models, and regulatory sources, offering advanced analyses on persistence and mobility. It provides stakeholders with access to curated data and tools for independent replication.

The cleanventory incorporates data from 38 files representing 25 inventories across 13 trade markets, encompassing over 990,000 entries, including 225,000 unique CAS Registry Numbers. Data cleaning and harmonization were critical for compatibility and quality. Structural information was inferred using CAS numbers and names via APIs (PubChem, CAS Common Chemistry, CCTE CompTox, and NCI/CADD), returning InChI strings ranked for probability using a weighted consensus approach. Persistence and mobility data were curated from scientific literature, QSAR models, and REACH dossiers and analysed using probabilistic Bayesian models to quantify uncertainties.

The cleanventory includes over 344,000 unique InChI strings, with 126,000 identified as "most probable" structures. Initial assessments suggest that approximately 50% and 75% of entries exhibit persistence and mobility potential, respectively. The cleanventory aligns with the EU Chemical Strategy for Sustainability, supporting regulatory processes with transparent, high-quality data.

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7.02.P-We530 Inter-Species Extrapolation of Toxicokinetic Data via Mechanistic Models: Benchmarking in Small Mammals

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Environmental risk assessment necessitates the evaluation of numerous species that cannot be directly tested due to ethical and resource limitations. At the same time, the efforts under the 3R (reduce, replace, refine) principle emphasize reducing in vivo tests with the help of in vitro and in silico methods. In addition to the 3R goals, such approaches have the potential to allow a more mechanistically informed (next generation) risk assessment. Such mechanistic approaches will require mechanistic computational models to allow extrapolations from in vitro to in vivo, from lab to field, and between species. One tool to perform such extrapolations are physiologically-based kinetic (PBK) models. PBK models are complex toxicokinetic (TK) models that describe the body as a multitude of interacting organs. PBK models allow for the integration of in vitro data to predict the TK profile and to put in vitro effect data into a realistic exposure context.

This study aimed to assess the data requirements and performance of PBK models in extrapolating TK data among small mammals. We parameterized PBK models for three mammal species (*Rattus norvegicus*, *Mus musculus*, *Oryctolagus cuniculus*) and performed cross-species extrapolations for six compounds, all six possible reference-target species combinations, and systematically omitting available (in vitro) data.

The results demonstrate a significant improvement in prediction performance over a body-weight scaled model across all data sources, with clearance data contributing most to performance. A limited in vitro dataset can yield successful extrapolation, comparable to direct fitting of target data. The analysis also indicated that performance can be reduced when extrapolating beyond the dose range of the reference data set or when saturation effects are apparent.

We established a benchmarking framework for evaluating the species-extrapolation performance and data requirements of PBK models. The findings indicate that effective extrapolation is achievable with limited in vitro data. This framework can be expanded to include additional compounds, species, and administration routes.

7.02.P-We531 Mapping Contaminated Sites and Potential Sources to Understand the Extent of Known and Potential Contamination in the EU

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The EU faces a critical challenge with an estimated 2.8 million contaminated sites requiring environmental action. Despite this, there are still significant gaps in data on key issues such as soil pollution. To address this issue, an EU-wide harmonized database is required to effectively identify contaminated sites and plan remediation and restoration.

In this project, data from national inventories, the scientific literature, reports from national authorities, and other literature on pollution activities and associated contaminants are collected to map contaminated sites for per- and polyfluoroalkyl substances (PFASs), petroleum and coal based (PETCO) substances, metals, and organochlorine and -bromine compounds. The collected information includes key descriptors of the sites, such as contaminant identity, concentration, and nearby sources. Additional descriptors associated with the contamination such as soil properties, land use, depth of the contamination, and size of the contaminated area are also collected. To locate the source of the contamination, information from the European Pollutant Release and Transfer Register (E-PRTR) is used. The E-PRTR contains information on activities and reported emission volumes. However, it cannot be concluded from the available emission data that the activity always leads to emissions of certain substances. The extracted data show that from all facilities that report a certain activity, only a very low percentage (mostly < 5%) report emissions of the same substance. However, the emission data from the E-PRTR are still used in the project to identify potentially contaminated sites.

The final produced maps and the collected data can be used for spatial planning, risk assessment and socio-economic analysis for evaluating and prioritizing soil remediation strategies. These maps will therefore be an important tool to achieve healthy soils in the future. In the poster we will show the data collected thus far, address challenges in obtaining the data and present contaminated hot spots in Europe.

7.02.P-We532 Enhancing Environmental Exposure Assessments: A Tiered Approach for Downstream Users in the REACH Context, a Case Study with the Cosmetic Industry

Harald Streicher, Diego Robles and Lale Carstensen, Beiersdorf AG, Germany

Given that REACH is a chemicals regulation, it is well understood that environmental exposure assessment was predominantly designed for chemical manufacturers, employing a total tonnage approach that aggregates data across diverse sectors. While essential for regulatory compliance, this method often fails to provide actionable insights for downstream users, such as cosmetic manufacturers, who need specific exposure data relevant to their products. For instance, UV filters used in cosmetics may also be found in coatings and plastics, each with distinct exposure routes. This highlights the inadequacy of a one-size-fits-all tonnage approach, particularly for substances washed down the drain after use.

To address these limitations, we propose a tiered framework for environmental exposure assessment tailored to the cosmetic industry. The first tier focuses on an industry sector approach, concentrating on realistic volumes of down-the-drain exposure specific to cosmetics. However, obtaining accurate tonnage data can be challenging. Therefore, we recommend a company-specific tier, allowing individual companies to assess their actual usage tonnages. This enables precise calculations of respective environmental impact and facilitates tracking changes in ingredient use over time, serving as a basis for estimating industry sector volumes based on market share.

Moreover, the REACH total tonnage approach yields average exposure predictions for Europe, which may not reflect local realities. The diversity of river catchments complicates the correlation between predicted environmental concentrations (PEC) and actual measured concentrations (MEC). To enhance accuracy, we advocate integrating data from human safety assessments, such as the SCCS Notes of Guidance, which provide average usage values for cosmetic categories. By combining these values with default effluent data and environmental fate models like SIMPLETREAT, we can achieve a more accurate picture of environmental concentrations, further refined by incorporating country-specific dilution factors and wastewater treatment facility percentages.

In conclusion, we advocate for a comprehensive exposure assessment strategy that incorporates total tonnage, industry sector, and company-specific approaches. This multilevel approach enables downstream users to make ingredient decisions that align with environmental safety goals.

7.02.P-We533 Croplife Europe: Problem Formulation Approach for Environmental Testing Strategy of Biochemical Plant Protection Products

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Biochemicals such as plant extracts, RNA, peptides and microbial metabolites have been gaining importance for their use as crop protection products. They are recognized to provide a sustainable alternative to synthetic pesticides and provide farmers with valuable options for pest management strategies.

However, there are concerns that the approval of biochemicals in the EU has been limited, the main challenges include the extensive data requirements and use of 1107/2009 which was not specifically designed for biochemical products. Croplife Europe have therefore developed a proposal for the environmental risk assessments of biochemicals, based on a problem formulation approach which allows

for a more targeted and adaptable testing strategy. Instead of establishing minimum risk in all possible aspects of environmental risk the scope is to use all available information on the biochemical to characterize the product e.g. natural occurrence, mode of action, stability etc to identify potential hazards to non-target species, and to determine if there is a viable exposure pathway to those species based on its intended application method.

Using a tiered approach to the assessment, with data added to address any uncertainty for non-target effects or to refine the exposure assessment, the aim of this assessment is to ensure safe use can be confirmed using a targeted and efficient data set. This method provides a transparent approach using dependable scientific assessment. CLE aims to promote the innovation of novel biopesticides is encouraged with an adaptable yet robust regulatory process.

7.02.P-We534 Promises and Challenges on the Way to the Next Generation of Environmental Risk Assessment for Pesticides

Nina Hallmark and Tobias Pamminger, Environmental Safety, Bayer AG, Germany

Globally, environmental risk assessment (ERA) methodology is deeply rooted in the long-standing societal concern about the hazards of manmade chemicals and their potential risks to the environment.

Traditional ERA frameworks are based on laboratory-based hazard characterization data, typically combined with worst-case environmental exposure estimates. The methods used to develop these data have continuously evolved e.g., in vitro toxicity testing, ecological modelling, environmental fate modelling, satellite supported landscape mapping, and many others. Therefore, as we progress into the 21st century and further information types and quantity become available it becomes clear that combined with changes in societal expectations linked to broader information access e.g., via on-line sources, an updated risk assessment paradigm is needed.

his new ERA framework must be fit for contemporary challenges and expectations as the underlying societal drive for minimal unintended impact on human or environmental health and safety is increasing too.

The paradigm of risk assessment applied to chemical safety is changing. A next generation risk assessment paradigm is emerging, led by the cosmetics sector and focused on human safety. For environmental safety assessment application of NGRA (NGeRA), the human safety approach cannot be copied as the level of data and subsequent assessment for individual organisms in needs to be extrapolated across vastly different and numerous species. Instead, the focus will be on creating models and frameworks that can be adapted and applied across various species and scenarios. This approach allows for a more scalable and efficient assessment process, ensuring that environmental safety standards are met without the need for exhaustive individual-level data for every species.

This is an opportune time for discussion within the safety science and research communities to design and implement the tools to enable the use of the best science available in chemical safety assessments. And then convince the regulating authorities.

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7.02.P-We535 Next Generation Risk Assessment Case Study on Biocides Regulated under K-BPR: A Role of NAMs Data in Regulatory Decision-Making

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New Approach Methodologies (NAMs) include a variety of non-animal testing techniques such as in silico, in chemico, and in vitro methods. Despite their potential, NAMs data are often underutilized in regulatory decisions due to concerns about reliability. This study investigates the role of NAMs in assessing the hazard potential of biocides regulated under the Consumer Chemical Products and Biocides Safety Act (K-BPR). A systematic analysis was conducted using ToxCast/Tox21 bioactivity data and non-standardized data from literature focusing on human toxicity endpoints relevant to K-BPR regulations. By comparing hazard assessment outcomes from the EU-BPR report, where biocides had been previously evaluated, with NAMs data from ToxCast/Tox21 and the non-standardized data, several inconsistencies were identified. For instance, while the EU-BPR classified azole fungicides as non-carcinogenic and non-genotoxic, multiple in vitro studies indicated evidence of DNA damage. Similarly, developmental toxicity concerns highlighted by the EU-BPR were corroborated by findings of cytotoxicity in human placental cells. Moreover, although rat studies reported no neurotoxicity, NAMs data revealed positive cytotoxicity

in human neuroblastoma cells. This case study underscores the importance of integrating NAMs data into chemical hazard assessments to avoid underestimating risks. It highlights the potential of NAMs to enhance the accuracy of chemical risk assessments and advocates for their broader acceptance in regulatory frameworks.

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7.02.P-We536 EMBL AgriData Platform - Delivering Safe Food And Environmental Health

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Introduction: Feeding a growing global population amidst climate change is a critical challenge. Climate resilient crops are increasingly required, together with environmentally benign pest and disease control strategies that are in synergy with the agricultural ecosystem. The European Bioinformatics Institute s (EMBL-EBI) AgriData Platform aims to optimize agricultural research and development (R&D) through effective management of agricultural biodata.

AgriData Platform Features: With EMBL-EBI data resources at its foundation, the ADP will boast several core features essential to its success. Firstly, it will integrate diverse data types beyond genetics, including environmental factors like climate, soil quality (type, ecological diversity at the microbial level, its nutritional potential for growing crops etc), farming practices and safety of pesticides. This holistic approach ensures a comprehensive understanding of the challenges and opportunities for plant breeding, crop protection and agronomy. Secondly, it will provide user-friendly computational tools to analyse and interpret integrated data, catering to users with varying levels of expertise. Thirdly, the platform will be designed to be sustainable and scalable, accommodating the incorporation of proprietary data from commercial organisations. Lastly, it will engender collaboration between industry (across agri-tech R&D sectors) and academia, leveraging EMBL-EBI s expertise of building data platforms in the biomedical sector for Open Targets.

Conclusions: EMBL-EBI s AgriData Platform aims to address global food security challenges through optimised agricultural research and data-driven innovation. ADP aims to create sustainable, high-quality data resources and computational tools, fostering cross-industry collaboration and driving impactful solutions in agricultural R&D.

7.03.P Health and Well-Being Effects of Blue Spaces: The Ocean-Human Health Nexus in an Ocean Under Stress

7.03.P-Tu499 From the Blue Gym to Blue Health, Blue Communities and Blue Prescriptions: 15 Years of Promoting the Oceans for Human Health

Mathew White¹ and Lora Fleming², (1)University of Vienna, Austria, (2)University of Exeter, United Kingdom

Much of the focus of the field of Oceans and Human Health (OHH) has been on the risks and threats to public health including a wide array of chemical and microbial pollution, invasive species, harmful algal blooms, sea level rise, flooding etc. But our seas and oceans also have numerous benefits for human health and well-being; there are reasons why coastal regions are the most visited holiday destinations globally, and why house prices on the coast are often higher than inland. While fully recognising the risks, it is also important not to lose sight of the benefits, not least because one the main drivers of environmental protection is the desire to avoid benefit loss. This talk will present highlights from a 15yr research programme focusing on these benefits, especially the relatively intangible benefits to mental health and well-being. It will begin by detailing the Blue Gym project which was the first to show that living near the coast was associated with better health across several countries in the world and to identify the main four pathways why this might be: a) greater levels of physical exercise; b) better social and community relations; c) lower levels of stress and mental distress; and d) better biopsychosocial resilience. It will then introduce three multi-country, multi-disciplinary, European Union funded (H2020/Horizon Europe) research projects: BlueHealth, SOPHIE (Seas Oceans and Public Health in Europe), and RESONATE (Building individual and community RESilience thrOugh NATurE-based therapies); and one UK Govt. funded project working with coastal communities in South East Asia (Blue Communities). In all four

projects attempts are made to understand the benefits to public health from interactions with our seas and oceans, alongside the more widely discussed risks. For instance, while a traditional approach may look at the number of hospital admissions from exposure to marine pollutants, we balance this with estimates of health care savings arising from population levels of physical activity at the coast. As well as providing systematic multi-method evidence of the various benefits of living near and interacting with the sea, the talk will also cover a range of individual, local and societal level interventions to improve access and exposure to (safe) marine settings to support and improve population health especially among more vulnerable communities.

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7.03.P-Tu500 The Coastal Exposome: Investigating the Link Between Sea Spray Aerosols and Human Health Benefits

Silke Lambert, Jan G. Bourgois, Greet Cardon, Colin Janssen and Jana Asselman, Ghent University, Belgium

Oceans produce sea spray aerosols (SSAs) by bursting bubbles from breaking waves. SSAs are a complex mixture of inorganic salts, marine microbiota and bioactive molecules. Inhalation of low concentrations of these marine microbiota and bioactive molecules by acute and/or chronic exposure to coastal environments can potentially interact with cell signaling pathways, leading to positive health effects. Our research group previously demonstrated potential beneficial health effects of SSAs in vitro through inhibition of the Mammalian Target of Rapamycin (mTOR) pathway. mTOR is a key cell signaling pathway, regulating cell proliferation, autophagy and apoptosis. It has been associated with cancer, arthritis, insuline resistance and osteoporosis. At present, it remains unclear however which marine microbiota and bioactive molecules have an effect on the mTOR pathway and to what extent they are able to interact with the mTOR pathway in vivo.

7.03.P-Tu501 The Social Structuring of Coastal Visitation Behavior

Alexander Hooyberg¹, Stefaan De Henauw¹, Gert Everaert², Nathalie Michels¹ and Henk Roose¹, (1)Ghent University, Belgium, (2)Flanders Marine Institute (VLIZ), Belgium

Half of all recreational tourism involves a coastal destination, and increasing evidence shows that coastal nature provides various benefits for health and well-being. Yet, the diverse behaviors at the coast have remained poorly mapped. Following Bourdieu's distinction theory, we hypothesized the existence of key structuring patterns in visitors' activities and social interactions at the coast and clear associations with visitors' demographic, socio-economic, and health characteristics. Using data collected in Belgium from 2022 (N=1302), we found four structuring dimensions based on visit frequency, preference for beaches vs. built environments, visits with family vs. friends/alone, and socialization vs. exploration. We also segmented five types of visitors: Salty Socializers, Family Trippers, Singles In The City, Senior Foodies, and Lone Roamers. Visitors' behavior varied with age, household situation, and level of social support. Our findings illuminate the pivotal role of citizens' social capital in understanding visitors' exposome at the coast and repercussions for health.

7.03.P-Tu502 Assessing the Emotional Impact of Landscape Features on Well-being: An Online Video Experiment Across Flanders

Yangyang Shi, Filip Raes and Ben Somers, KU Leuven, Belgium

Both green and blue spaces play crucial roles in supporting emotional well-being. However, the specific landscape features that contribute to these benefits remain unclear, with most research relying on site-specific experiments. To bridge these gaps, our study employs an online video experiment across Flanders to investigate how the proportion of green-blue spaces and soundscapes shape individual's emotional health. Participants (n=571) are recruited through online platforms, social networks, and offline posters. Each participant is randomly assigned to watch one of the designed videos, assess their emotional states both before and after viewing, and complete a follow-up questionnaire. We address two primary research questions: (1) How do visual and auditory characteristics independently and interactively influence individual's emotional states in urban and natural environments? (2) Does combining blue and green

spaces provide greater emotional benefits than a single type of space? We have registered this study on OSF (<https://osf.io/d4fzu>) and are currently in the data collection phase. Our data analysis will examine the following hypotheses:

H1: Participants exposed to a natural environment with natural sounds will experience significantly greatest emotional benefits, including an increase in positive affect, a decrease in negative affect, and a reduction in worry, compared to all other conditions (i.e. natural environments with urban noise or no sound, urban or office environments with urban noise, natural sounds, or no sound).

H2: In urban environments, exposure to natural sounds will result in significantly greater emotional benefits (as defined in H1) compared to exposure to urban noise or no sound.

H3: Natural sounds in an urban environment will result in significantly greater emotional benefits (as defined in H1) than urban sounds in a natural environment.

H4: Participants exposed to environments with a combination of blue and green spaces will report significantly greater emotional benefits (as defined in H1) compared to those exposed to environments with only blue or only green spaces.

This study aims to deepen scientific understanding of how specific landscape features impact emotional health, which contributes valuable insights for urban planning. The results may help shape city planning guidelines to optimize urban spaces and therefore enhance the mental health benefits associated with green and blue spaces.

7.03.P-Tu503 Ocean and Health: A Paradigm to Face the Challenge of Sustainable Development Goals (SDGs) at Plentzia Marine Station (PiE-UPV/EHU)

Manu Soto, Oihane Diaz de Cerio, Ibon Cancio, Nestor Etxebarria and Ionan Marigomez, University of the Basque Country (UPV/EHU), Spain

Plentzia Marine Station (PiE-UPV/EHU) is integrated in the European Research Infrastructure European Marine Biological Resource Centre (EMBRC, www.embrc.eu), established in 2018 with a mission to provide research services to foster fundamental and applied marine biology and ecology research and to promote blue biotechnologies. The motto of PiE-UPV/EHU Ocean and health is related to the holistic concept of One health, globally and recently adopted by scientific and political communities. It implies that connections between human and living organisms and their shared environment are essential for well-being. Health must be understood comprehensively in order to protect the environment and humans. For instance, health means healthy food and aquaculture is relevant in the European Farm to Fork strategy. One of the fields with most potential for improving sustainability of aquaculture is the research of innovative feeds based on alternative ingredients and on non-carnivorous target species. PiE-UPV/EHU together with other entities in the Basque Country is conducting research towards on the intensive culturing of the omnivorous mugilid thicklip grey mullet *Chelon labrosus* (AKURA project) since 2017. In addition, SLOW ALGA Project initiated in 2021 explores the characteristics of algae from the Basque Coast to explore the possibility of creating km 0 products to support the Basque gastronomic ecosystem and generate healthy food with high nutritional value. In addition, research groups from the Basque Science & Technology Network (Neiker, BC3, UPV/EHU, PiE-UPV/EHU) are integrated in the Joint Research Laboratory "Environmental Antibiotic Resistance" developing joint activities on the underlying One Health paradigm. Within the EMBRC and UN Ocean's Decade endorsed activity EMO-BON PiE-UPV/EHU is also involved on pan-european marine biodiversity monitoring program based on omics techniques. Within these activities, the aim is to share experience and scientific interests and protocols to offer holistic solutions to problems that affect the one-health context. The main contribution comes from the European project BlueAdapt, led by BC3, and the Spanish project HOBE, led by both PiE-UPV/EHU and BC3, working together to establish Plentzia Bay as One Health Observatory (or Living Lab) where the land-sea transition can be studied with special attention to aspects such as the horizontal gene transfer of antibiotic resistance genes between land and marine microorganisms.

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7.03.P-Tu504 Using Wearable Technology to Assess Physiological and Cognitive Health Effects of Coastal Walking Exposure in Older Adults: A Field Experiment

Julia Kinet¹, Sara Vandamme¹, Colin Janssen¹, Greet Cardon¹, Mirko Petrovic², Gert Everaert³ and Jana Asselman¹, (1) Ghent University, Belgium, (2) Ghent University Hospital, Belgium, (3) Ocean & human health, Flanders Marine Institute (VLIZ), Belgium

With global life expectancy rising, the incidence of chronic health conditions, such as cardiovascular disease and Alzheimer's disease, is increasing, creating a significant healthcare burden. Exposure to natural environments as a preventive health strategy shows promise for positive health effects, particularly in reducing stress and improving cognitive function. While the health effects of green spaces are well-documented, the physiological effects of coastal environments for older adults remain underexplored. This issue is especially relevant in Belgium, where a large proportion of the coastal population exceeds the age of 65. This study addresses these knowledge gaps by examining the physiological and cognitive effects of coastal walking in older adults using wearable technology. In a randomized cross-over design, 60 participants, aged 60 and older, each complete two 30-minute walks, one in a coastal environment and one in an urban environment in Ostend, with a one-week interval between sessions. Each walk is preceded by 15 minutes of sedentary exposure. Continuous measurements of heart rate variability (HRV) and electrodermal activity (EDA) are collected using the EmbracePlus wristband and the Polar H10 chestband, and GPS coordinates and movements are recorded during the walks. Salivary cortisol levels are assessed at four time points, and cognitive performance is evaluated before and after each exposure using the d2 Test of Attention and the Symbol Digit Modalities Test. Furthermore, self-reported mental health data is gathered pre- and post-exposure. In the first phase of this study, a pilot experiment with 15 participants (ages 21-56, 53% female) was conducted to test and refine the protocol. The pilot study revealed a possible familiarity bias: the urban walk had a more pronounced stress-reducing effect than the coastal walk, potentially due to participants' greater familiarity with the urban environment. Building on these findings, the current study controls for this familiarity bias, while also increasing the sample size and employing more suitable outdoor wearables. It is hypothesized that exposure to the coastal natural environment will have a more pronounced positive effect on (physiological) stress and cognitive function in older adults compared to the urban environment. This study will contribute to the growing body of research on nature-based interventions, offering potential strategies to improve health outcomes in ageing populations.

7.03.P-Tu505 The Effect of Coastal Exposure and the Role of Activation on Emotions and Emotion Regulation: an Innovative Experimental Design

Elias De Craene¹, Gert Everaert², Ann Buysse³ and Filip Raes⁴, (1)Flanders Marine Institute (VLIZ); Ghent University; KU Leuven, Belgium, (2)Flanders Marine Institute (VLIZ), Belgium, (3)Ghent University, Belgium, (4)KU Leuven, Belgium

Coastal environments form a unique natural landscape associated with mental health benefits, where people often report greater happiness by the coast compared to other environments. Furthermore, emotions, emotion regulation strategies (ERSs), and activation, are all linked to changes in mental health outcomes. Emotions and ERSs are an important component to the recent nature-based biopsychosocial resilience theory, a fundamental framework describing how nature exposure can promote well-being. Therefore, to gain a better understanding of the link between mental health and the coast, a significant step forward lies in examining the role of experienced emotions, ERSs, and activation in this context, an area that remains underexplored. This study aims to address this research gap through an experimental design using virtual exposure. Four conditions will be compared by combining the following elements: exposure to either a coastal or urban environment, with or without activation (resulting in a 2x2 design). Exposure will take place in a controlled setting within an immersive projection room featuring 360° videos and accompanying audio of a coastal (or urban) environment, providing an innovative solution to the limitations of picture experiments or virtual reality biases. This method should allow controlling for random, real-life disturbances, while maintaining a higher level of ecological validity compared to less immersive methods. The activation manipulation will also align with real-life contexts. Prior to exposure, a sadness induction will be conducted for standardization, and, post-exposure, emotions and ERSs will be assessed to compare outcomes across conditions. It is hypothesized that participants will experience more positive emotions and use more adaptive ERSs in the coastal exposure and activation conditions (main effects) and that this effect will be even stronger for coastal exposure and activation together (interaction effect). Ethical approval will be sought from the local ethics committee, and the study will be pre-registered to ensure transparency. Based on power calculations, the target sample size will be set. It is anticipated this study will contribute to the understanding of how exposure to a coastal environment influences emotional responses and ERSs. As such, findings may inform policymakers in designing environments that support mental well-being and may increase awareness of the potential health benefits of the coast.

Track 8. Special Sessions

8.01 - Biodiversity and Chemical Hazard/Risk Assessment: Regulatory Frameworks, Integrative Approaches and Research for a Sustainable Future

8.01.T-01 Introductory Remarks

Romana Hornek-Gausterer, Environment Agency Austria, Austria

8.01.T-02 Panellist group 1: flash presentations

8.01.T-03 Discussion with Panellists group 1 and Audience

Romana Hornek-Gausterer, Environment Agency Austria, Austria

1. What is the current state of ERA and biodiversity?

Discussion on the current state of chemical ERA methodologies and whether they adequately address biodiversity.

2. To which extent do we understand biodiversity and its interrelated threats?

Discussion on the conceptual understanding of threats and the extent of our knowledge about them.

8.01.T-04 Panellist group 2: flash presentations

8.01.T-05 Discussion with Panellists group 2 and Audience

Romana Hornek-Gausterer, Environment Agency Austria, Austria

1. What knowledge gaps and barriers hinder effective ERA and management of biodiversity loss?

Discussion on key knowledge gaps and gaps in ERA methodologies and biodiversity management, including availability/knowledge of tools to be applied when assessing the effects on biodiversity, limited understanding of cumulative effects of chemical mixtures, challenges to extrapolate effects from laboratory to field and across species, and interactions between species and non-chemical and emerging pollutants. Challenges in predicting and mitigating biodiversity loss will also be discussed.

2. What new, innovative methodologies exist to enhance biodiversity conservation?

Discussion on biodiversity research and methodologies tailored to the evaluation of the impact on biodiversity status in ERA (prospective, retrospective or their integration, dependent on the regulatory need). Special emphasis is put on innovative solutions and methodologies, such as New Approach Methodologies (NAMs). Exploring the vision for next-generation ERA (NG-ERA) to enhance biodiversity conservation.

8.01.T-06 Concluding Remarks

8.02 - Bridging the Scientific and Regulatory Gap Towards Effective Chemical Safety Assessment

8.02.T-01 Introductory Remarks

Marlene Ågerstrand, Stockholm University, Stockholm, Sweden

8.02.T-02 Metabolic disruptors: achievements and challenges

Juliette Legler, Institute for Risk Assessment Sciences, Utrecht University, Netherlands

Overview of methodological challenges and achievements in characterising emerging hazards, and the subsequent regulatory challenges such as method validation and uptake of new methods into chemicals legislation.

8.02.T-03 Managing risks posed by legacy pharmaceuticals under the Water Framework Directive

Lina Gunnarsson Kearney, Swedish Agency for Marine and Water Management, Sweden

Challenges for an efficient and effective assessment process, focusing on the difficulties of filling data gaps with academic data. This includes the reliability and relevance of studies, including the population relevance of study endpoints.

8.02.T-04 Panel discussion

Jane Muncke, Food Packaging Forum Foundation, Switzerland

Main discussion questions:

- What structural way forward is needed to make the most of the tri-lateral (Academia-Industry-Regulators) interaction and to fill the “regulatory gap” with scientific innovation/scientific state of art?
- How to allow and facilitate the use of new methodology and non-standard endpoints in the regulatory setting?

Sub - topic 1: How to address the main hurdles, seen from the tri-lateral perspective?

- How to address hurdles on regulatory acceptance and the fit into the current regulatory landscape (i.e. regulatory perspective)?
- What hurdles to take from an academic perspective: funding, structural sustainability?
- What challenges to overcome from an industry perspective: legal certainty and technological preparedness?

Sub - topic 2: How to best integrate new scientific evidence into regulatory frameworks?

- How to use non-standard endpoints and studies in a regulatory setting?
- How to capture relevant population effects: realism in terms of methodological challenges, cost-effectiveness and feasibility?

Questions from the audience

8.02.T-05 Concluding Remarks

Simone Rizzuto, European Food Safety Authority (EFSA), Parma, Italy

8.03 - European Commission Roadmap Towards Phasing Out Animal Testing for Chemical Safety Assessments: State of the Process and How Can SETAC Community Contribute

8.03.T-01 Introductory Remarks

Georg Streck, European Commission, Belgium

8.03.T-02 EC commission road map process and EPAA ESA work

Georg Streck, European Commission, Belgium

8.03.T-03 EPAA ESA structure and work

Jose V. Tarazona¹ and Bruno Campos², (1)Institute of Health Carlos III (ISCIII), Spain, (2)Unilever, Bedford, United Kingdom

8.03.T-04 Discussion with Audience: Recommendations for the EC Roadmap

Georg Streck, European Commission - DG GROW, Belgium

Discussion with audience: recommendations for the EC roadmap focussing on seven relevant categories: Fish acute, Bioaccumulation, Fish Chronic, Endocrine disruption, Birds and Mammals (wildlife), Validation, New Environmental Safety Paradigm

Each category will start with leading expert very briefly presenting the recommendations and asking the audience to score each category on following criteria:

*Recommendation relevance

*Recommendation priority

*Any other recommendation missing

8.03.T-05 Concluding Remarks

Georg Streck, European Commission, Brussels, Belgium

8.04 - Next-Gen Environmental Science: Collaborative Networks for a Sustainable Future

8.04.T-01 Introductory Remarks

Markus Schmitz, *Evolutionary Ecology & Environmental Toxicology, Goethe University Frankfurt, Frankfurt, Germany*

8.04.T-02 Introduction to SETAC SAC

Markus Schmitz¹ and **Micha Wehrli**², (1)*Evolutionary Ecology & Environmental Toxicology, Goethe University Frankfurt, Frankfurt, Germany*, (2)*Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland*

8.04.T-03 Introduction to ASPIS Academy

Shaleen Glasgow

8.04.T-04 Introduction to PARC Junior Community

Maria Tannous, *Daei, ANSES, French Agency for Food, Environmental and Occupational Health & Safety, France*

8.04.T-05 PARC II: Tissue-specific responses to a binary mixture of bisphenol A substitutes in zebrafish embryos: a focus on two estrogenic and metabolic bioassays

Florian Geffroy, *INERIS, Ecotoxicology of Substances and Environment (ESMI) Unit / Université Paris Cité, Inserm UMR-S 1124, France*

Mixtures of chemicals, notably endocrine-disrupting chemicals (EDCs), represent a major issue as current regulations often underestimate their risks by assessing individual substances only. Most approaches for evaluating EDCs mixtures rely on cellular models, thereby precluding to take into account the complexity of whole-organism systems and capture potential tissue-specific interactions. In this study, we aimed to address this gap by using transgenic zebrafish embryo models to assess the effects of a binary mixture of bisphenol A substitutes, bisphenol B (BPB) and bisphenol C (BPC), on estrogenic and metabolic pathways in two distinct target organs, the brain and the intestine. Through in vivo fluorescence imaging, we observed that both BPB and BPC induced the expression of the estrogen-regulated aromatase B gene (*cyp19a1b*) in the brain, as well as the intestinal expression of *cyp3a65*, each in a concentration-dependent manner. For each substance and target gene, EC50 values were derived and used to set-up an experimental ray design for binary mixtures (5 mixture ratios chosen by considering relative potency and 5 exposure levels around the EC50). The observed tissue-specific effects of these mixtures will be compared with predictions from the commonly used “concentration addition” model for our two differentially regulated target genes. These findings could reveal new insights on mixture interactions in whole-organism systems, across differentially regulated genes expressed in two distinct organs, supporting a better hazard assessment of EDCs mixtures.

8.04.T-06 PARC I: Integrated Risk Assessment for Pyrethroids: A “Proof of Concept” approach for toxicokinetics’ supported NAM-based toxicodynamics

Ana Fernandez Agudo, *Institute of Health Carlos III (ISCIII), Spain*

Pyrethroids are synthetic insecticides widely used in agriculture and households, raising health concerns from potential ingestion, inhalation, and skin exposure. While intended to target insect nervous systems, pyrethroids may also present neurotoxic and bioaccumulative risks to human organs such as the liver, kidneys, brain, and lungs. Traditional risk assessments often apply Paracelsus’s 16th-century principle, “the dose makes the poison,” using a deterministic threshold model to determine safe exposure limits. However, emerging research challenges this paradigm, revealing that the single-substance assessment and the lack of accountability of the individual variabilities are not realistic scenarios, suggesting a need for more nuanced assessments that account for bioaccumulation and complex exposure scenarios.

This study integrates toxicokinetics and toxicodynamics using PKSim software and ToxCast data to assess six pyrethroids by organ-specific bioactivity and pathway categorization, comparing results with in vivo NOAEL benchmarks. This approach includes in vitro-in vivo comparisons to improve predictions of tissue-specific toxicity and reflects a paradigm shift toward NGRA, which incorporates probabilistic, mechanism-based models.

Combining toxicokinetics and toxicodynamics, the study identified organ-specific toxicity patterns, with simulations showing bioaccumulation risks within cells despite safe interstitial levels. PKSim and Tox21 model comparisons validated the reliability of in vitro methods in NGRA.

NGRA’s integrated, probabilistic framework refines traditional models by moving beyond fixed

thresholds, supporting more precise, organ-specific assessments and addressing limitations of Paracelsus's principle. This approach, incorporating both aggregate and mechanistic insights, offers a robust basis for chemical safety evaluations that align with real-world exposure complexities.

8.04.T-07 APSIS Academy: Advancing NAMs-based DNT risk assessment through refined biokinetic models

Susana Proenca, *esqLABS GmbH, Netherlands*

New approach methods (NAMs) are crucial for addressing the challenges in developmental neurotoxicity (DNT) risk assessment posed by species differences and knowledge gaps. While the OECD is currently evaluating their application for DNT, there is a lack of detailed recommendations for developing and evaluating the necessary kinetic models.

The ONTOX project aims to improve in silico biokinetic models for DNT to advance the predictive potential of NAMs-based DNT risk assessment. For in vitro kinetics, an in silico model was adapted from Armitage et al. (2021) and Fischer et al. (2017), incorporating both logKow-based and polyparameter linear free energy relationships-based quantitative structure-activity relationships (QSARs) and allowing simulation of repeated exposure. Simulations of approximately 100 DNT chemicals in a typical DNT in vitro system, using this model, revealed 10-fold lower free concentration than nominal concentration for 15 % of the chemicals, highlighting the need for accurate dosimetry.

Further, comparisons of pregnancy and neonates physiologically-based kinetic (PBK) models across PK-Sim, httk, and Simulation Plus platforms revealed similarities (C_{max} within 10-fold) for most chemicals but specific differences particularly for very polar and highly lipophilic chemicals, where PK-Sim exhibited relatively lower C_{max} predictions. For these chemical domains, kinetic predictions are being compared to available human in vivo data, and where mispredictions are identified, the PBK model or parameterization are being adjusted accordingly.

The final aim of this study is to integrate DNT effect data with the adjusted in vitro kinetic and PBK models and evaluate different quantitative in vitro in vivo extrapolation strategies.

8.04.T-08 Closing the Circle: How SETAC and Early Career Networks Shape Environmental Science

Markus Brinkmann, *Toxicology Centre, University of Saskatchewan, Saskatoon, Canada*

As the final speaker of this session, Dr. Markus Brinkmann, Director of the Toxicology Centre at the University of Saskatchewan, Canada, will reflect on his experiences with SETAC and its Student Advisory Council. As current co-chair of the Bioaccumulation Science Interest Group (BSIG) and as a former SAC Chair and co-host of the second Young Environmental Scientists (YES) Meeting in Landau, Dr. Brinkmann will share anecdotes and personal insights into the role of early career networks in supporting professional growth and fostering collaboration.

His talk will explore the opportunities and challenges of engaging with such networks and highlight how these experiences have influenced his own career. Dr. Brinkmann will also discuss how SETAC and its affiliated groups can continue to promote connections and provide valuable support for early career scientists navigating their paths in environmental science.

As an independent contribution to the session, this talk will set the stage for the concluding discussion. It offers a perspective on how established SETAC structures and emerging early career networks can build on their strengths to empower the next generation of researchers.

8.04.T-09 Panel discussion: SETAC ESR and other ESR associations: networking & cooperation

Markus Schmitz, *Evolutionary Ecology & Environmental Toxicology, Goethe University Frankfurt, Frankfurt, Germany*

8.04.T-10 Concluding Remarks

8.05 - The European Green Deal (Chemicals Strategy for Sustainability): Leveraging the AI Power to Advance Biodiversity Protection

8.05.T-01 Introductory Remarks

Michelle Bloor, *Scotlands Rural College, United Kingdom*

8.05.T-02 Anthropogenic chemicals as underestimated drivers of biodiversity loss

Ksenia J Groh, *Environmental Toxicology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland*

Anthropogenic chemicals as underestimated drivers of biodiversity loss

8.05.T-04 Chemicals management and IPBES biodiversity assessment from the prospective of the business-lead author.

Marie-Helene Enrici, *Sustainability, Solvay, France*

8.05.T-06 Possible roles of mechanistic modelling in assessing chemicals as a causal factor in biodiversity loss.

Andreas Focks, *Institute of Mathematics, University of Osnabrueck, Osnabrück, Germany*

8.05.T-08 Ranking questions and moderated panel discussion

Michelle Bloor, *Scotlands Rural College, United Kingdom*

8.05.T-09 Concluding Remarks

8.06 - The Planetary Boundaries for Biodiversity in the Context of Safe and Sustainable by Design

8.06.T-01 Introductory Remarks

Annegaaïke Leopold, *Calidris Environment BV, Netherlands*

8.06.T-02 Introduction to the cross-cutting issues with regard to Carrying Capacity, SSbD, Governance, Planetary Boundaries for biodiversity

Leo Posthuma, *Centre DMG, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands*

8.06.T-03 Pitch talks: What is essential and innovative? - A few expert perspectives

8.06.T-04 Discussion with Panellists and Audience

Annegaaïke Leopold, *Calidris Environment BV, Netherlands*

Key questions to be addressed:

- What is the most urgent innovation and connection between SSbD and Planetary Boundaries that we need to make.
- What is needed to improve current regulations

8.06.T-05 Concluding Remarks

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